Development of a Screening Programme to Select Salt Tolerant Genotypes of *Eucalyptus microtheca* F.Muell and *Acacia nilotica* (L.) Willd. ex Del.

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DEDICATION

Dedicated to my father Sheikh Aziz-ur-Rehman and my mother Kurshid Aziz with love, respect and recognition

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ABSTRACT

This study was designed to develop a technique to select putative resistant genotypes of trees for use in silviculture of saline areas within the tropics. *Sinapis alba (L.)* (White Mustard) was used at the development stage: seedlings were subjected to different concentrations of NaCl (34, 102, 205 and 410 mol m^{-3}) plus control and survival of the seedlings at high salt concentrations was considered to be a suitable criterion for selection of resistant genotypes within the population. There was no survival at the highest level of salinity (410 mol m^{-3}).

The presence of some survivors at high salinity levels suggested the existence of genetic variability within the population for salt tolerance. The seedlings selected at the first stage of the study (screening test) were exposed again to salt stress in order to investigate their growth performance. Classical plant growth analysis was used to derive values of relative growth rate ($\overline{RGR}$), net assimilation rate ($\overline{NAR}$), leaf area ratio ($\overline{LAR}$), specific leaf area ($\overline{SLA}$) and leaf weight ratio ($\overline{LWR}$). All these growth parameters except NAR decreased with increase in salinity. There was no significant difference in NAR between the treatments.

The pilot study was followed by an investigation on *Eucalyptus microtheca* (F. Muel) and *Acacia nilotica (L.)* Willd. ex Del. using the same methods, putative resistant genotypes within the population of the species were screened out through subjecting them to different concentrations of NaCl (102, 205 and 410 mol m^{-3}) and control. The survival percentage decreased with increase in salinity and some individuals survived even at high concentrations. This survivorship implies existence of genetic variability for salt tolerance within the population of the two tree species.

In order to test if survivors were more resistant than the population average to salinity, seedlings survived at each level of NaCl concentration were subsequently exposed to different concentrations of salinity (102, 205 and 410 mol m^{-3}) and control. The classical growth analysis was repeated to derive the values of $\overline{RGR}$, $\overline{NAR}$, $\overline{LAR}$, $\overline{SLA}$ and $\overline{LWR}$.

One of the main effects of increasing salinity was a linear decrease in RGR for both the species, due to a decrease in NAR and not LAR (unlike *Sinapis alba*).

The growth performance of the selected individuals was better than the unselected ones in terms of relative growth rate (RGR) but they grew better even when they were in a salt-free medium. It was concluded that selection of the individuals seems to be partly for vigour rather than salt tolerance, although it seemed that the selected individuals displayed less sensitivity of net assimilation rate to an increase in salinity.

Irrespective of whether the selection is for vigour or salt tolerance, it is likely to be beneficial if applied at the nursery stage. The selected individuals can be expected to outgrow the non-selected by a considerable margin, as a small gain in RGR implies a large gain in biomass.

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CHAPTER 1

SALT-AFFECTED SOILS - BACKGROUND AND THEIR UTILIZATION WITH PARTICULAR REFERENCE TO PAKISTAN

1.1 INTRODUCTION:

Land degradation is the decline in productive capacity of an ecosystem through adverse changes in the life-supporting processes of its climate,vegetation, soil or water resources. According to one estimate, of the 1449×10^6 ha of currently cropped land an additional 2000×10^6 ha of formerly productive land have been rendered unproductive through irreversible degradation (UNEP, 1986). FAO/UNEP (1983) estimated that at present 5-7 million ha of cultivated land (0.3 - 0.5%) are being lost every year through soil degradation. The projected loss by the year 2000 is feared to be 10 million ha annually (0.7% of the area presently cultivated).

Major causes of land degradation are large scale deforestation and the altering of vegetation caused by rapid increases in human population. Soil degradation, caused partly by deforestation and degradation of vegetation but mostly by soil misuse and over-exploitation, is a severe global problem. It includes physical processes - such as deterioration in soil structure, erosion and excessive inundation; and chemical processes such as the decline in soil fertility, adverse changes in salinity, acidity or alkalinity and the effects of toxic chemicals or pollutants (UNEP, 1982). According to IUCN (1977), an additional area of 12 million hectares suffers from serious soil degradation every year and a total of 2077 million ha of degraded land currently exist in tropical countries (Grainger, 1988, Table 1.1).

Forests are an essential resource for economic development. They provide lumber for housing, pulp for paper and biomass for fuel. Biomass energy is the primary fuel resource (about 80%) for the poor people of the world. Approximately half of the biomass is woody material, 33% crop residues and 17% dung (Pimentel *et al.*, 1986). But world-wide deforestation is running at about 11.6 million ha of land annually (FAO, 1982), most of it due to the growing need to increase agricultural production (Evans, 1982) for which about 10 million hectares of new land are needed every year (Pimentel and Hall, 1989). The rate of destruction of tropical forests has increased dramatically in recent years attaining an estimated rate of 20-50 hectares per minute (Leakey, 1986). At the same time, world demand for forest products is increasing in line with world population, to which

TABLE 1.1 : Area of Tropical Degraded Land (ha × 10^3)
(Source : Grainger, 1988)

Region	Logged forests	Forest fallows	Deforested watersheds	Desertified dry lands	All lands
Africa	38,956	59,292	3,126	740,900	842,274
Asia	53,574	58,770	56,494	748,000	916,838
Latin America	43,993	84,754	27,230	162,000	317,977
Total	136,523	202,816	86,850	1,650,900	2,077,089

it is directly related. According to projected estimates, the world population will double by the year 2000 (Freijka, 1973) and on the basis of the above predictions it is concluded that:

(a) world demand for wood will double, provided the per capita wood consumption remains constant;
(b) the use of wood as fuel and industrial raw material is likely to increase as non-renewable resources become scarcer or more expensive; and
(c) the per capita wood consumption is likely to rise if poor nations become less poor (Wood, 1976).

If the present trend of demand and supply continues an annual deficit of nearly 15000 × 10^6 m^3 is expected during the next 20 years; about 50 × 10^6 hectares of forest plantations are needed to maintain even present levels of consumption (El-Lakany, 1986). However, there are approximately 758 million ha of degraded tropical lands with the potential for forest replenishment. Of these, 418 million ha are in dry or mountain areas in need of afforestation or reforestation; 137 million ha are tropical rain forests requiring protected regeneration or silvicultural manipulation; and 203 million ha are forest fallows in the humid tropics requiring an adequate fallow period to be reforested (Grainger, 1988, Table 1.2).

Based on the previous forecasts of future demands for fuelwood and industrial wood, it is estimated that afforestation of a third of all degraded lands in mountain regions (26.8 million ha) and about a fifth of the degraded crop lands in the dry region

TABLE 1.2 : Area of degraded tropical lands with potential for forest replenishment

Region	Area (ha $\times$ 10^6)
Montane	87
Dry lands	331
Humid tropics, logged forests	137
Humid tropics, forest fallows	203
Total	758

(61.5 million ha) would prevent the projected fuel wood deficit in those regions by the year 2000 (Grainger, 1988).

1.2 SOIL SALINITY - A PROBLEM OF THE WORLD

Accumulation of excessive salts in the root zone to levels that are toxic to plant growth occurs in arid and semi-arid regions where the mean annual evaporation greatly exceeds the precipitation. In other words soil salinity occurs where the supply of salts from rock weathering, capillary rise, rainfall or flooding, for example, exceeds their removal by drainage, leaking or run-off. Climatic aridity, an important factor for salt enrichment is attributed to low precipitation, high evapotranspiration, high temperatures and low humidity (Landon, 1984). Saline soils are easily formed in regions where the ratio P/PET is less than 0.75 (where P is precipitation and PET is potential evapotranspiration; Pimentel and Hall, 1989).

Among the most commonly cited factors contributing to the degradation process is irrigated agriculture (Mabutt *et al.*, 1981 and Pimentel and Hall, 1989) which is often perceived as a way to improve crop production in arid areas. Huge investments in irrigated infrastructure by national governments and international lending agencies have been motivated by a variety of pressures to solve or mitigate immediate economic problems. Predominant among these is the need to increase crop yields, to provide more rural employment and to raise rural income. According to Kovda (1982) about

30-35 percent of the global irrigated land is affected with salinity and alkalinity and a large part of this area is in the tropics (Swaminathan, 1987).

The accumulated salts usually comprise chlorides, sulphates and carbonates of sodium, magnesium and calcium. Among these, sodium chloride is the predominant salt determining soil salinity followed by sodium carbonate or sodium sulphate and salt of magnesium (Chapman, 1966). Excessive salts hinder growth, not only by toxicity effects but also by reducing water availability through the action of osmotic pressure in water and nutrient uptake.

The FAO/UNESCO soil map of the world (FAO/UNESCO, 1971-81) classifies the salt-affected areas as:

(a) Solonchak soils, which have high concentrations of salts causing osmotic drought and hindrance to normal uptake of water and nutrients; and,

(b) Solonetz soils, which have sufficient exchangeable sodium to cause unfavourable physical conditions, toxicity and non-availability of micro-nutrients.

In addition there are saline and sodic phases of other areas. The salt-affected soils can be defined as follows (Dudal and Purnell, 1986);

(i) Solonchak Electrical Conductivity (EC_e) value > 15 dS m^{-1} (deciSiemens) in the upper 0.75-1.25 metres of the soil profile.

(ii) Saline phase EC_e values 4-15 dS m^{-1} in upper 1.0 metres.

(iii) Solonetz Exchangeable sodium percentage (ESP) > 15 in the upper 0.4 metres.

(iv) Sodic phase ESP 6-15 in the upper 1.0 metre.

A general interpretation of salt concentration and electrical conductivity of saturation extract (EC_e) with crop reaction is given in Table 1.3.

The total land area of the world is 13.2×10^9 hectares, of which saline soils cover a substantial portion: estimates vary from $400\text{-}950 \times 10^6$ hectares (Massoud, 1974) up to 1011×10^6 hectares (Dudal and Purnell, 1986). As many as 400 million hectares of the 1500 million hectares currently under cultivation have enough salts to reduce the agronomic potential of these areas (Mudie, 1974), excluding regions classified as arid lands (Flowers *et al.*, 1977). With the large areas of arid land it is possible that the total land area affected by elevated levels of salts exceeds 150 million hectares or approximately 40% of the 400×10^6 hectares of potentially arable land (Rains, 1979).

TABLE 1.3 : General interpretation of electrical conductivity of saturation extract (EC_e) values (milli Siemens/m)

USDA Soil Class	Designation	EC_e (mS m^{-1})	Total Salt Content (%)	Crop Reaction
0	Salt free	0-2 0-2	< 0-15	Salinity effects are mostly negligible Salinity effects are negligible except for the most sensitive plants
1	Slightly Saline	4-8	0.15-0.35	Yield of many crops restricted
2	Moderately Saline	8-15	0.35-0.65	Only tolerant crops yield satisfactorily
3	Strongly Saline	> 15	> 0.65	Only very tolerant crops yield satisfactorily

Salt-affected soils are widely distributed in different continents. The data in Table 1.4 show that an estimated 1011 × 10^6 hectares are presently affected with varying degrees of salinity, Asia and Australia having the largest areas.

Reclamation techniques which comprise flushing the salts through drainage and following appropriate cropping systems with balanced fertilizer applications can restore the productivity of some of the salt-affected soils. However, providing drainage to leach salts from the drainage basin is difficult. The salt temporarily leached from root zone, accumulate in the ground water and eventually into the rivers. Salts are then easily recycled to the root zone through irrigation.

Some of the irrigated areas of Australia, India, Pakistan, China and Middle-East are highly prone to developing salinity problems.

According to Mudie (1974), a total of 53 × 10^6 ha (about one quarter) of the world's irrigated land is salt-affected. Expansion of irrigated agriculture in arid and semi-arid regions is likely to aggravate the problem of salinization. Lack of adequate drainage has caused severe salinization in some countries. Worthington (1977) observed that salt-affected soils amount to 50% of the irrigated area in Iraq, 23% in Pakistan, 50% in Euphrates Valley of Syria, 30% in Egypt and more than 15% in Iran. Such salt-affected areas which are not fit to restore to agricultural production either due to technical reasons or lack of financial resources can possibly be used for

TABLE 1.4 : Distribution of Salt-affected Soils (× 10^6 ha) (Source : Dudal and Purnell, 1986)

Region	Solonchaks	Saline	Solonetz	Sodic Phase	Total	*Salt-affected soils (% of region)
Europe	2.3	13.7	7.9	56.6	80.5	4.6
North America	0.1	6.2	4.0	-	10.3	0.9
Central America	0.2	1.7	-	-	1.9	0.7
South America	10.5	58.9	14.8	45.0	129.2	7.6
Africa	43.6	47.7	1.9	5.4	98.6	3.5
South Asia	47.2	36.1	-	18.0	101.3	-
North & Central Asia	22.5	69.2	30.1	90.0	211.8	21.0
South-East Asia	-	20.0	-	-	20.0	-
Australia	16.6	0.8	38.1	301.9	357.4	42.3
Total	143.0	254.3	96.8	516.9	1011.0	

* after Massoud (1974)

establishment of forestry plantations. El-Lakany (1986) observed that the salt-affected land in arid and semi-arid areas could be used for the production of wood and fuel from salt-tolerant species. In addition, he considered that newly reclaimed saline areas (through chemical or engineering means) should not be subjected to the same type of land use: rather, it seems appropriate to set aside these environmentally degraded areas for forestry plantations. Alternatively, salt-tolerant trees could be integrated with agricultural crops on such areas in agroforestry systems to prevent or at least slow down the process of salinization. Many species of trees can grow adequately on these problem areas where most agricultural crops cannot. Also trees have a role in the desalinization of soil (El-Lakany, 1986) through their ameliorative effects such as improving soil structure and permeability (by the action of the root system), adding organic matter through leaf litter and root residues, producing carbonic acids through root activity and decay of organic matter, stimulating microbial activity and moderating the effects of climate (Yadav, 1975).

According to Swaminathan (1987) such environmentally degraded areas can often be reclaimed through agroforestry practices. Recently, throughout the tropics, agroforestry, social forestry and farm forestry are becoming popular. The selection of species for higher production in general, and their suitability for degraded areas in particular, needs to be intensified.

1.3 SALT-AFFECTED AREAS IN PAKISTAN

The geographical area of Pakistan including Azad Kashmir is 87.98 million hectares and its population at the 1986-87 census was 104.4 million. The average annual rate of population growth is about 3% which is among the highest in the world (Amjad and Khan, 1988). It is situated entirely to the north of the tropic of Cancer, has been included within tropical Asia since it comes under tropical and sub-tropical influences which are felt to be significant (FAO, 1982).

The climate of most of Pakistan is arid to semi-arid, having less than 200-500 mm of average annual precipitation. The distribution of precipitation varies widely in terms of both space and time. The average mean summer temperature is about 40°C with individual readings as high as 50°C. Potential evaporation is higher than precipitation by a factor of more than 22 (Sandhu and Qureshi, 1986).

The soils are generally moderately coarse to moderately fine in texture, calcareous, low in organic matter and alkaline. The cation exchange capacity ranges from 8-16 milli-equivalents per 100 grams of soil. Cultivation of agricultural crops is the major land use in Pakistan. The cultivated area amounts to 31.57 million hectares (36% of total area). The per capita availability of cultivable land is 0.302 hectares.

1.3.1 The Forest Base of Pakistan

Pakistan is a forest-poor country with only 4.74 million hectares of forests i.e. 5.4% of the total area of the country (87.98 million ha). Of this, 1.48 million ha (1.68% of total area) are productive forests (Table 1.5). This low forest cover compares unfavourably with several other countries of the region; Malaysia 64.5%, Sri Lanka 42.4%, India 23.7%, China 17.7% and Bangladesh 15.3%. The per capita forest area is a mere 0.045 ha compared with the world average of 1 ha.

About 37% of the total forest area is privately owned leaving 63% owned by the State. The area under forests in the country is decreasing with the increase in demand for forestry products from an increasing population. The estimated loss of forest area in Pakistan is 1.1% per year (Ichord, 1985) and about 2000 hectares of closed forest is being encroached upon every year (FAO, 1981).

TABLE 1.5 : Extent and distribution of forest area in Pakistan 1986-87 (Source : Amjad and Khan, 1988)

Province	Total land area (million ha)	Forest Area		Production Forest		Per Capita forest area (ha)
		Area (million ha)	% of total area	Area (million ha)	% of total area	
NWFP	10.17	1.41	13.9	0.26	2.6	0.088
Punjab	20.63	0.63	3.1	0.34	1.6	0.011
Sind	14.09	0.68	4.8	0.30	2.1	0.030
Baluchistam	34.72	0.72	2.1	0.00	0.0	0.138
Northern area	7.04	0.94	13.4	0.22	3.1	1.388
Azad Kashmir	1.33	0.36	27.1	0.36	27.1	0.151
Total	87.98	4.74	5.4	1.48	1.68	0.045

1.3.2 Supply and Demand of Wood

Because of the acute scarcity of wood and high prices, the annual per capita consumption of timber is about 0.0239 m^3 which is among the lowest in the world. For a population of 105 million in 1988, timber consumption works out to be 2.50 million m^3. Of this, 0.364 million m^3 were supplied by state controlled forests (i.e.

14.6%), 1.097 million m^3 (44%) by imports and the remaining 1.034 million m^3 (41.4%) by private farm lands (Amjad and Khan, 1988).

However, with increase in population the demand for timber and other forestry products will increase and the country will have to import more timber. Annual yield from forests is also very low i.e. 2 m^3 ha^{-1} for productive forests (Ichord, 1985) and with this low rate of production it does not seem possible that the country could cut its heavy imports of timber and other products. Rather it is expected that import of forestry products will increase with increase in demand. However, the yield from the forests can possibly be increased by intensifying the management through construction of infra-structure, mechanized harvesting and switching over to artificial regeneration instead of fully depending on natural regeneration.

Fuel wood is a vital component for survival, especially in rural areas for cooking and heating, accounting for as much as 90% of all wood consumed in Pakistan (Draper *et al.*, 1978). According to the Pakistan household census of 1980, 79% of the rural and 48% of the urban households use fuel wood for cooking i.e. so that overall fuel wood contributes as much as 70% of the total energy consumption in terms of housing units (Table 1.6).

TABLE 1.6 : Types of Energy used for Cooking by housing units, 1980
(Source : Population Census Organisation, 1982)

Fuel for Cooking	Housing units ($\times 10^6$)	%age
Wood	8810	70
Coal	87	1
Kerosene Oil	781	6
Gas	813	6
Electricity	11	-
Cow dung and agricultural residues	2086	17

This represents about 50% of the total energy requirements of the domestic sector of the country, the balance being provided by fossil fuel (13%), agricultural residues and cow dung (37%) (Pakistan Forest Institute, 1983).

The per capita consumption of firewood is estimated at 0.2 m^3 per annum (in addition the 0.02 m^3 timber already mentioned). With the population of 105 million for 1988, the annual consumption of firewood comes out to 21 million m^3 against an annual production of 2 million m^3 from state controlled forests. Pakistan Forest Institute (1983) has estimated that, by the year 2000, the country's fuel wood requirements are likely to increase to 30 million m^3 per annum.

While demand for fuel wood has been increasing, its overall resource base appears to be shrinking. While production from state forests is important, the production from private farmlands is even more important as this accounts for over 90% of the total fuel wood production. In order to bridge the prospective gap between supply and demand of timber and firewood by the end of the year 2000 (Table 1.7), about one million hectares of land is required to be brought under forestry plantations.

TABLE 1.7 : Projected demand and supply of timber and fuelwood by the end of year 2000 (Population 145.1216 million) (Source : Amjad and Khan, 1988)

Particular	Timber (million m^3)	Fuelwood (million m^3)	Total (million m^3)
Demand	3.468	29.024	32.492
Supply			
State Forests	0.364	2.000	2.364
Farmlands	1.034	18.800	19.834
Deficit	2.070	8.224	10.294

Obviously it is undesirable to put good fertile agricultural land under forests: the likely areas available for forest plantations could be environmentally degraded land such as saline or alkaline soils or eroded areas.

1.3.3 Utilization of Salt-Affected Areas

Large scale irrigation projects on the river Indus, Swat, Kabul and Jehlum have been the basis for agricultural development in Pakistan. The total irrigated area in Pakistan increased from 12.99 million hectares in 1971-72 to 14.79 million hectares in 1981-82 (Biswas, 1987). While the increase in irrigated area was 13.8% the quantity of irrigating water available at the farmgate increased by 35% during the same period. The additional water was utilized by:

(i) over-irrigation by the farmers;
(ii) changes in the cropping pattern e.g. the growing of high water consumption crops; and
(iii) use of additional cultivable land that had not been possible before within the possible reach of irrigation canals. (Biswas, 1987)

More frequent irrigation or over-irrigation and extension of the canal network allows vastly increased seepage from canals as well as increased infiltration from fields. As a result, the water table rises nearer to the surface where enhanced evaporation occurs, resulting in waterlogging and increased salinity. The twin menace of waterlogging and salinity has plagued the agriculture of Pakistan and is one of the major causes of low agricultural production in the country. Saline and sodic soils occur naturally in arid and semi-arid regions like Pakistan and the problem is accentuated with the introduction of artificial irrigation. The estimates of salt-affected areas in Pakistan differ widely because of the different criteria used for survey by different government organisations. According to Snelgrove (1967) already 1.9 million ha have been lost to salinity and another 4.5 million have developed saline patches since the inauguration of canals in 1930. Mohammed (1978) reported that some 10 out of the 15 million hectares of canal irrigated land are becoming saline and waterlogged and thousands of hectares are going out of production ever year. According to Speece (1982) nearly half of Pakistan's irrigated land is affected to some degree of waterlogging and/or salinization while Bokhari (1980) estimated that 40,000 hectares of fertile irrigated land was going out of production every year owing to salinity and waterlogging. Toenniessen (1984) has reported that in Pakistan, 80% of the irrigated land is affected to some degree of salinization. Carlston (1953) identified three main features as being responsible for waterlogging and salinity:

(a) leakage from irrigation and distribution systems;

(b) infiltration of applied irrigation water; and

(c) increased recharge from run-off due to the obstruction of natural drainage courses.

Serious attempts have been made to eradicate soil salinity during the past two decades but the results of these have somewhat mixed. The present status of surface salinity profile is shown in Table 1.8.

TABLE 1.8 : Surface Salinity Profile (000 ha) in Pakistan (Source : Biswas, 1987)

Salt free	Slightly Saline	Moderately Saline	Strongly Saline	Misc. land type	Total
12,032 (72%)	1838 (11%)	1002 (6%)	1336 (8%)	501 (3%)	16,709 (100%)

1.3.4 Characteristics of Saline Soils of Pakistan

The salient features of the saline soils of Pakistan are given by Choudhry (1972) and Choudhry *et al.* (1978) as follows:

(i) they contain mostly sodium chloride ($NaCl$) and sodium sulphate ($Na_2 SO_4$) salts;

(ii) the concentration of cations is directly proportional to the total soluble salts and usually decreases with an increase in depth; predominant cations are Na, Ca and Mg, with K present only in minor quantities;

(iii) soluble sodium predominates among the cations in all the profiles, while among the soluble anions chlorides and sulphates occur in higher amounts than carbonates and bicarbonates; and

(iv) the electrical conductivity of soil varies from 4 to 20 mS cm^{-1} and ESP $< 15\%$ to $> 50\%$. The pH varies from 6.9 to 9.2.

1.3.5 Summary

Accumulation of salts in the soil surface is characteristic of arid and semi-arid regions. The salinization occurs both naturally (fossil salinity) and also as a consequence of poor drainage of irrigation water (secondary salinity). Billions of dollars are being spent on projects aimed at reclamation of affected areas. The economic costs of waterlogging and salinity in Pakistan are estimated at US \$25 ha^{-1} yr^{-1} totalling US \$ 140×10^{6} yr^{-1} (Sandhu and Qureshi, 1986).

Taking into consideration the huge expenditure involved in the hydrological and chemical approaches of reclamation, saline agriculture (the growing of salt-tolerant crops and halophytes e.g. grasses and other commercial crops on salt-affected areas where normal cropping is not possible and/or is not economical) seems to be the most appropriate and economic answer to the problem (Sandhu, 1988). The reclamation of saline soils so that any crop can be cultivated proves to be an expensive exercise. The alternative approach of saline agriculture, involving utilization of salt-affected soils and their biological reclamation by growing salt-tolerant trees, shrubs and grasses appears to be feasible. If it is not possible to re-create the conditions that existed before the onset of salinity then it is possible to find plants which can successfully grow under existing saline conditions and are capable of showing minimum depression in yield. Since most crop plants are comparatively sensitive to salinity the planting of multi-purpose salt-tolerant tree species may offer an attractive alternative for economic utilization of such lands (Ansari *et al.*, 1988).

Thus, environmentally degraded areas can possibly be put to their best use by raising forestry plantation. Quite recently social forestry programmes have been launched in the country with the primary objective of raising fuelwood plantations on privately owned farmlands, either in the form of wood lots or mixed with agricultural crops. The aim of these programmes is to develop and expand the forest base of the country for meeting the increasing demand for timber and firewood. Most of the land available for raising of woodlots is affected by some degree of salinity including the areas along roads, canals and railway tracks.

All these ecological, economic and strategic factors further enhance the possibility of making use of salt-affected areas for raising forestry plantations in the country.

1.4 AIMS OF THE THESIS

In view of the importance of salinity as a limitation to forestry in Pakistan, it was decided to concentrate attention on aspects of the salinity problems:

(1) To review the literature on afforestation of salt-affected areas, with particular emphasis on previous work in the Indian subcontinent (Chapter 2).

(2) To review the literature on salt tolerance in plants (Chapter 3).

(3) To develop a screening test, to establish an experimental technique which might subsequently be used in the Forestry Departments in Pakistan: to begin with, mustard *Sinapis alba* (L.) was used as the test plant (Chapter 4), later it was possible to obtain seeds from Pakistan of *Eucalyptus microtheca* F. Muel. and *Acacia nilotica* (L.) Willd. ex Del. (Chapters 5 & 6).

CHAPTER 2

AFFORESTATION OF SALT-AFFECTED AREAS

2.1 INTRODUCTION

Two approaches to afforestation of saline soils seem possible: (1) growing salt tolerant trees without any reclamation, (2) first improving the soil conditions through chemical amendments or engineering works before less salt resistant but economically more valuable crops are grown. Since the reclamation of salt affected soils through improvement of soil is a costly operation attention is to be focussed on growing appropriate salt resistant tree species, using suitable planting techniques which vary according to the nature and intensity of salinity.

2.2 TREE PLANTING AS A METHOD OF RECLAMATION

Tree planting has repeatedly been suggested as a mean of controlling salinity (Morris, Jenkin and Collett,1981; Greenwood, 1978; Carland and Duff,1981; Anderson,1982 and Khan and Yadav,1962). Many scientists feel that even economically feasible engineering schemes cannot eliminate salt from saline environments but can only minimize its effects.Trees might have a role in the de-salinization of soils (El-Lakany,1986). It is considered that trees reclaim the saline soils through their different functions (Chaturvedi,1985) :

(1) by providing shade and mulch which reduce the rate of evaporation from the soil surface;
(2) by absorbing part of the salt present in the soil;
(3) by transpiring more water as compared to agricultural crops, thus lowering the water table;
(4) by addition of leaf litter and other dead plant materials which on decomposition improve the physical and chemical composition of the soil; and,
(5) by increasing the permeability of the soil through the action of roots.

Certain forest tree species are more tolerant to the saline conditions as compared to agricultural crops (Chaturvedi,1985). Yadav (1975) reviewed the literature on the use of forest trees, agricultural crops and grasses for improvement of saline and alkaline soils (mostly in India), and described the growth of several tree species on these soils and the ameliorative effects of the well known salt tolerant tree *Prosopis juliflora* . Also Shah (1957) after field observations reported that the *Prosopis juliflora* helps to reduce salinity and alkalinity of the soil after a prolonged occupation and in course of time, makes it cultivable, for some time at least. Yadav and Singh (1970) observed a decrease in the value of pH and soluble salt content and an increase in the status of organic matter and nitrogen in the upper 15 cm layer of soil under *Prosopis juliflora* .

The succulent leaves of *Calotropis* allow a high potential of salt uptake and its cultivation offers de-salinization possibilities of saline soils (Boyko and Boyko,1966). According to Sandhu and Qureshi (1986) roots have various beneficial effects on saline soils, including the release of carbon dioxide which may lead to increase in the dissolution of carbonates.

De-salinization normally takes place under the tree growth in salt-affected soils (Kratinin,1967 and Nigunova,1972). The work of Vadiunea (1964) showed that the tree growth substantially reduces the salt content of soil. Some of the tree species absorb and extrude large amounts of salt from soil and thus mitigate the problem of salinity to some extent (Boyko and Boyko,1966 and Smith,1960).

The work at Banthra Research Station, Lucknow (India) shows the ameliorative effects of the tree canopy cover by reducing soil pH and exchangeable sodium and increasing organic carbon. A pronounced increase in Ca/Na ratio under dense canopies was recorded (Khanduja *et al.*, 1987).

Gill and Abrol (1986) reported the ameliorative effects of *Acacia nilotica* and *Eucalyptus tereticornis* through decrease in soil pH and electrical conductivity. The soil organic carbon content increased under both *Acacia* and *Eucalyptus* and the water infiltration rates of the soil also improved. Temperatures were reduced in the summer and increased in winter. Migunova (1972) also found changes in salinity of soil profiles under various broad leaved species: trees had a pronounced effect on salinity through the changes they brought about in water, air and thermal regimes of the soils.

The role of trees in reclamation of salt-affected soils is widely recognized. Rehabilitation of sites through afforestation is a slow but steady process and is cheaper than reclamation through engineering or chemical measures. It is therefore clear that salt tolerant trees could make the best possible use of salt affected soils. Emphasis should be placed on the selection of suitable tree species and the identification of resistant genotypes among species' populations which give minimum depression in yield when grown in saline conditions.

2.3 CHOICE OF SPECIES

The salt tolerance of forest tree species differs greatly (Bangash,1977; Blake, 1981; Chaturvedi,1985; El-Lakany,1986; El-Lakany and Luard,1982; Malik and Sheikh,1983; Omran, 1986; Pepper and Craig,1986 and Yadav and Singh,1970) and there is ample scope for selection of species according to the nature of the soil and other habitat conditions. Correctly matching the species to the site conditions is very important in environmently degraded areas, since there is substantial variation in the site conditions of such areas and the requirements of tree species. For example drainage patterns vary from place to place even on smaller trial plots, and these changing conditions affect the establishment and growth behaviour of the plants.

Morris and Thomson (1983) have described the following three criteria for selecting a tree species for planting on saline soils:

(i) adaptation to saline site conditions- the ability to grow and maintain healthy conditions over an extended period;
(ii) the capacity to transpire large quantities of water, including the ability to tap ground water where appropriate; and
(iii) the provision of economic and other benefits in addition to the lowering of water table and control of salinity.

Although some understanding of the salt tolerance of a few tree species is available, rather little is known about the growth performance of many of these species on saline areas. However, the choice of species for a particular area will depend on the criteria laid down by Morris and Thomson (ibid).

One of the well known salt tolerant tree species among *Acacias* is *Acacia arabica* (syn. *A. nilotica*) which is widely planted on salt-affected areas. Field studies conducted by Yadav (1980) showed that *Acacia nilotica* and *Prosopis juliflora* can grow on salt affected areas and attained height equal to those on normal soils provided minor soil amendments like gypsum, farmyard manure and NP fertilizer are added.

Survival and growth performance of 27 species of trees and shrubs planted in saline sand were studied by Lavrinenko and Volkov (1973) and the results after six years indicated that the best tree species were *Populus alba, Ulmus pumila, Elaeagnus angustifolia, Robinia pseudoacacia* and *Ailanthus altissima* .

Field trials on saline and alkaline soils conducted by Bhargava (1951) in Madhya Pradesh (India) showed that *Acacia nilotica, Prosopis juliflora* and *Azadirachta indica* were suitable. Bangash (1977) found that *Zizyphus jujuba, Albizia lebbek* and *Acacia* *nilotica* were the most salt tolerant species. Tsing *et al.* (1956) reported that *Azadirachta indica, Ailanthus altissima* and *Sapium sebiferus* showed the least sensitivity to high levels of salinity. Also it was found that stem cuttings of *Tamarix chinensis* and root cuttings of *Ulmus pumila* were highly tolerant to salt. Singh *et al.* (1986) in field studies in India found that *Acacia nilotica* grows well on moderately saline soils.

In Pakistan, field studies of salt-affected soils revealed a good performance of *Tamarix aphylla* and *Casuarina equisetifolia* . The observations of Popova (1957) indicated that the following tree species are tolerant of salinity (in decreasing order): *Tamarix ramosissima, Robinia pseudoacacia, Ulmus Spp., Fraxinus pensylvanica, var. lanceolata, Morus alba, Prunus armeniaca, Quercus* and *Acer species.*

For tropical regions, the Indian Forest Research Institute at Dehra Dun has summarized its observations as follows (Boyko, 1966):

(i) saline soils containing over 0.16% soluble salts (electrical conductivity values of about 2.5 mS m^{-1}) and possessing a hard-pan of stiff clay do not support tree or shrub species;

(ii) saline soils containing less than 0.16% soluble salt to a depth of 90 cm, with no clay or kankar pan, may have a scattered growth of *Acacia arabica (syn. A. nilotica), Salvadora oleoides, Tamarix species, Capparis decidua, Zizyphus species, Azadirachta indica, Albizia lebbek, Calotropis procera* and *Prosopis spicigera* ;

(iii) saline soils with a high water table but without a hard-pan, shallower than 75 cm depth, may support *Prosopis juliflora, Acacia nilotica, Acacia leucophloea, Capparis horrida, C. decidua* and *Salvadora oleoides* ;

(iv) saline sodic soils with efflorescences of salts may not support any tree;

(v) when the salt content in the surface horizon is less than 0.16% and a clay or kankar pan is deeper than 75 cm , the tree species which occur are *Acacia nilotica, Salvadora oleoides, Zizyphus spp.* and *Prosopis spicigera* .

In general, conifers are more sensitive to salinity than deciduous trees (Boyko,1966). Trees which showed good tolerance under temperate environments include *Salix babylonica, Elaeaganus angustifolia* and *Ulmus pumila* (Synder *et al.*,1940).

Dalbergia sissoo which is one of the most economically valuable species, widely planted under agroforestry systems and in irrigated plantations in the Indian subcontinent, can grow and withstand moderate salinity if once established (El-Lakany,1986).

In western Australia, planting on saline areas includes a variety of trees, *Eucalyptus sargentii, E. platypus, E. spathulata, E. camaldulensis, E. gracilis, E.kondininensis, Casuarina glauca, Tamarix articulata* and *Callitris glauca* (Hart, 1972). Hillis and Brown (1978) added *E. gomphocephala, E. rudis* and *E. microtheca* In the Negev region of Israel, *Eucalyptus camaldulensis* and *Pinus halepensis* were found to be the most tolerant on chloride solonchak soils having electrical conductivity between 12-17 mS cm^{-1} (Binder-Berhava and Ramati,1967). Gates and Brown (1988) have reported the potential of *Prosopis cineraria* and *Acacia tortilis* for their tolerance to salts. In north east Russia, Zelenin (1976) in a trial started in 1962 on salt affected soils found *Ailanthus altissima* to be a promising species for dry and saline conditions. Also, in the Sahara, *Eucalyptus camaldulensis, Acacia nilotica, Casurina torbulosa, Cupresses sempervirons* and various *Tamarix* have been reported to give good results under saline water irrigation (Karschon, 1966).

On the basis of what little information is available about the salt tolerance of tree species, choice of species is governed among many factors by the nature and amount of salt, relative proportion of ions, physical condition of the soil and its moisture status. Above all, the economic utility of the products is one of the important factors influencing the choice of species for afforesting any site. The relative growth of a species on such degraded areas must be considered and the species with comparatively better growth performance are to be preferred. Possibly multi purpose trees with nitrogen fixing ability will help in the establishment of plantations because saline areas are usually deficient in nitrogen.

2.3.1 Trees of Particular Interest

2.3.1.1 *EUCALYPTUS SPECIES*

The existence of a great number of species of *Eucalyptus* in enormously diverse habitats enables the selection of species and seed source for almost any environmental conditions including high salinity. Most of the *Eucalyptus species* are fast growing and have useful products.The considerable number of *Eucalyptus species* known to be salt tolerant provides a greater choice for selection of species depending upon soil conditions.

Blake (1981) screened 52 species and sub species of *Eucalyptus* for salt tolerance. As a result 11 species survived NaCl concentrations of 300 mol m^{-3} to 400 mol m^{-3} (high tolerance) including *Eucalyptus woodwardii, E. tereticornis, E. camaldulensis, E. calophylla, E. erythrocorys, E. largiflorens, E. grossa, E. incrassata, E. neglecta, E. globulus* and *E. lehmannii*. Also 18 species of *Eucalyptus* were identified to be moderately salt tolerant (200 to 300 mol m^{-3})*.

Among the list of highly salt tolerance *Eucalyptus camaldulensis* and *E. tereticornis* are widely planted in forest plantations and agroforestry practices in Pakistan.The studies in other parts of the world also recognized the potential of *Eucalyptus* species for their salt tolerance (Hart,1972; Karschon and Zohar, 1975; Malik and Sheikh, 1983; Radhi,1985; Moezel *et al.*,1988 and Sands,1981). *Eucalyptus microtheca* is the next important species, known to produce one of the strongest and hardest timbers in the world. It is said to be resistant to drought, high temperature and salinity/alkalinity and considered to be suited for shelterbelts and windbreaks being windfirm and free from insect and fungal pests (National Academy of Sciences,1980). In Sudan, irrigated plantations of *E. microtheca* have been established on salt-affected soils (Jackson, 1977). In Israel and Kuwait *Eucalyptus camaldulensis, E. microtheca, E. tereticornis, E. gomphocephala and E. robusta* have been found to grow on saline areas (Yadav, 1980).

2.3.1.2 CASUARINA SPECIES

Casuarina species are an excellent source of fuelwood. Some of the *Casuarina* species grow naturally on saline soils (Doran and Hall,1981) e.g. *C. glauca* is usually found close to brackish water on saline soils and *C. obesa* is known to grow naturally on salt-affected soils in western Australia.

Casuarina equisetifolia is a coastal variety which grows on the sea shores and is known to tolerate salt spray. It has root nodules containing nitrogen fixing actinomycete micro-organisms and is therefore not dependant on soil nitrogen.

Under experimental conditions Moezel *et al.*, (1988) found that *C. obesa* was the most tolerant species to saline water-logged conditions, with no seedling death and a lower reduction in growth compared to *Eucalyptus species*. Exclusion of Na^+ and

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* elsewhere in the thesis units of conductivity are employed. It has been found experimentally that the relationship between concentration of NaCl mixed in Hoagland nutrient solution and conductivity was 1 dSm^{-1} = 8 mol m^{-3}.

Cl^-, together with the production of specialized roots are suggested as the probable factors relating to its high seedling tolerance of saline waterlogged conditions.
El-Lakany and Luard (1982), in an experiment using solution culture of NaCl, found that *C. glauca, C. obesa* and *C. equsitifolia* are most tolerant as these survived at NaCl concentrations up to 550 mol m^{-3}. Among these *C. equsitifolia* is a well known species in India and Pakistan, considered to be suitable for planting on different sites particularly salt-affected areas.

2.3.1.3 OTHER IMPORTANT SPECIES

A variety of other important tree species are known for their tolerance to salt and being planted on saline areas in different parts of the world. The list of different tree species considered to be suitable for saline areas is quite long and includes species from the following genera: *Acacia, Prosopis, Albizzia, Robinea, Dalbergia, Parkinsonia, Leucaena, Azadirachta,* and *Salvadora,* with others of minor significance. However, the growth performance of many species included in these genera have not been fully investigated. Studies conducted for afforestation include the following species which could be useful for the conditions of Pakistan; *Acacia auriculiformis, Acacia catechu, Acacia nilotica, Acacia tortilis, Albizia lebbek, Albizia procera, Azadirachta indica, Dalbergia sissoo, Gleditsia triacanthos, Melia azadirachta, Parkinsonia aculeata, Pongamia pinnata, Prosopis cineraria (syn. Prosopis spicigera), Prosopis juliflora, Robinia pseudoacacia, Tamarix aphylla, Tamarix articulata and Terminalia arjuna.*

2.4 AFFORESTATION OF SALT-AFFECTED SITES

The edaphic and climatic features of salt-affected sites which need to be considered include the following:

(i) clay soils with low permeability,
(ii) limited availability of moisture;
(iii) toxicity due to the presence of high salt concentrations;
(iv) waterlogging and rise in water table (i.e. sites with poor drainage)
(v) very dry and hard conditions during the dry season;
(vi) hot winds, common in summer;
(viii) frost, usually during winter (the recurrence interval for severe frost is 4-5 years) whereby plants are damaged through frost lift;

(x) on certain sites the water available is brackish ground water.

In Pakistan the rainy seasons are very distinctive and conspicuous (winter and summer) and these are the normal times of planting. But this time of the year is the worse season from drainage point of view. The root system of the plants takes 2-6 weeks to establish on new sites depending upon the prevailing edaphic and climatic factors. However, during this period the roots of the plants are not fully active and if any waterlogging occurs during this time the roots are liable to rot. For this reason, the rainy season is not the correct time to plant up such sites.

At the same time planting is not advisable during hot summer months because the hot winds and high solar irradiance increase the rate of transpiration and winter is not suitable, as most species are affected by frost damage (i.e. frost lifting).

The most suitable time for planting on salt-affected sites is just after the rainy season when waterlogged conditions disappear. However, it will be an additional advantage if the species selected have the ability to withstand temporary waterlogging and have a superficial root system or the ability to adjust its root system from tap roots to surface roots. In addition, of course the species should be tolerant to drought. Preferably the species should not be palatable in its juvenile stage otherwise it will be liable to severe damage by cattle, sheep and goats. Saline areas are always deficient in nitrogen and organic matter and therefore a nitrogen fixing species is desirable.

2.4.1 Establishment Techniques - Ground Preparation

For successful tree plantations on salt-affected sites, selection of the proper method of soil working is of utmost importance. Any planting technique must meet the following requirements:

(i) a suitable mass of loose soil amenable to rapid proliferation of the root system;

(ii) an appropriate micro-relief of the soil mass to facilitate leaching of excessive salts to avoid a build up in the concentration of salts;

(iii) maximum soil moisture retention, particularly during periods of stress;

(iv) maintenance of fertility status; if necessary fertilizer and/or organic manure should be applied; and

(v) perforation of any compacted material present in the profile.

In order to achieve some of these requirements, the salt-affected soils are improved by different methods, depending on the condition of the site. These methods include;

- improvement of drainage through deep ripping
- lowering of watertable by suction pumps
- adding soil amendments like gypsum, sulphur and pyrite to reduce the salt toxicity
- use of fertilizer, green manure or other organic matter. (Ghosh, 1985)

During the dry spell of the year, the newly planted seedlings require to be mulched to prevent loss of water which will help to keep the concentration of salt low.

In many plantation trials, planting mounds up to 50 cm high are often made to combat temporary waterlogging conditions. However, the disadvantages of planting on mounds are that plants dessiccate during the periods of drought, since the root system is generally confined to the mounds, and also that plants are susceptible to windthrow particularly when their crowns are developed.

Chaturvedi (1985) has suggested that planting on salt-affected sites should be done in pits (90 cm deep with cross-section of 60cm x 60cm) at a spacing of about 2 metres and in order to get better survival, the pits being dug well before the time of planting and left unfilled for some time. When the soil has weathered well it should be back filled after mixing with farmyard manure or leaf litter. Abrol and Sandhu (1981) recommended planting in holes (15 cm in diameter and 120 cm deep) which were first filled with a mixture of 7-8 kg farmyard manure, 5 kg gypsum and original soil and then planted up. They reported 100% survival and good initial growth of *Eucalyptus* and *Acacia* seedlings planted in such holes.

Trench and ridge methods have also been considered most suitable (Shrivastava, *et al.*,1988). One of the advantages of this method is that heavy concentrations of salinity in the soil is reduced through leaching, thereby increasing the survival percentage and providing comparatively better growing conditions than planting in pits. Yadav and Singh (1970) after field trials on alkali soils found about 50% decrease in amount of soluble salts in the soils of the ridge. Ridges also maintain salt-free conditions, promote root proliferation and retard evaporation. Another advantage of this method is that rain water is utilized to the maximum possible extent. The spacement of trenches and ridges and their specification depends on the type of soil, climatic conditions, species to be grown, availability of irrigation facilities and the stocking density required. The results of field trials in Pakistan indicate that,with some site preparation (deep ploughing, use of *Sesbania aegyptiaca* as green manure, trenching, surface irrigation and fertilizer treatment), species such as *Acacia nilotica, Populus euphratica, Eucalyptus camaldulensis, E. robusta. E. rudis* can be grown on waterlogged areas which are not too highly saline (Sheikh, 1974).

The effects of salinity on plants varies depending upon its stage of development-sensitivity may be quite different during germination than at a later stage of life. Kling (1954) emphasized the importance of the initiation of photosynthetic activity in the seedlings before planting as a factor in increasing their osmotic pressure and salt tolerance.

The soil ground preparation techniques used in afforestation trials on salt-affected areas are summarized in Table 2.1: in most of the cases application of soil amendments are considered to be necessary.

TABLE 2.1 : Summary of soil ground preparation techniques for salt-affected areas (source: Shrivastava et al., 1988)

	Soil working/planting techniques	Country
1.	Ploughing 35 cm deep with mulch layer of chernozem soil about 20 cm thick	USSR
2.	Deep ploughing	USSR
3.	Loosening of soil after every good shower	India
4.	Continuous trench: 30 cm × 30 cm × 50 cm and 60 cm × 60 cm × 90 cm	India
5.	Trench and ridge	India
6.	Raised mounds	India
7.	Low ridges and channel (ridge 2.4 to 2.7 m apart, irrigation through channels between ridges)	Sudan
8.	Deep pits filled with fertile soil	India
9.	Pits and trenches and ridge with or without irrigation	Kuwait
10.	Staggered interrupted trenches	India

2.4.2 Planting/Sowing

On salt-affected areas sowing of seeds generally fails because the salt accumulates around the tender newly germinated seedlings and absorb the soil moisture (Chatervedi, 1985). Planting of nursery raised plants has few advantages over direct sowing of seed in the field except that sowing is comparatively cheap. Plants are usually kept in the nursery for 1-2 years and due to better care, are healthy and vigorous which gives better results when planted in the field. However, handling and carriage of plants from nursery to planting site play a vital role in determining success of the plantation. Great care is required to avoid unnecessary damage or shock to the plants.

2.4.3 Spacing

Spacing is a very important consideration in planting especially for commercial considerations. Organic matter is deficient on saline sites and root development is lateral. Consequently close spacing or high density planting is not likely to succeed. Although it is also desirable to cover the site quickly high density plantations are not desirable because they require higher initial investment. Therefore a compromise is needed, keeping in view both the condition of the site and requirement of the species.

2.4.4 Irrigation

Irrigation is an essential input in afforesting salt affected sites in arid or semi-arid areas. The salt concentration in the soil increases during the summer months specially when hot winds blow. Salts come up on the surface and dessiccation increases. Irrigation helps to keep the temperature down and provides the much needed moisture to plants in order to maintain leaf turgidity.

The best form of irrigation is through a sprinkler or trickle system. Surface irrigation is usually wasteful because there is a tendency to over irrigate. Any water that accumulates on the surface for several days deteriorates in quality and due to accumulation of salts, becomes toxic. Therefore, irrigation should be light and frequent, although no irrigation is usually needed during the rainy season.

2.4.5 Summary

Utilization of salt-affected soils for afforestation appears to be a promising form of land use because of increasing pressure on good soils for food production, fast exhaustion of firewood and timber resources and the need for healthy maintenance of an agro-ecological system. There can be one or combination of few approaches including hydrological/engineering, chemical and growing salt-tolerant plants to reclaim these environmentally degraded lands. Among these, growing trees on these soils independently or integrated with agricultural crops in an agro-forestry system has the advantage over other approaches being comparatively cheaper in cost.

Management of these soils for successful afforestation requires amelioration of the limited root zone for initial establishment and rapid growth. Thus, a special site preparation is generally considered necessary because tree planting procedures followed for the normal or good soils do not hold good for inhospitable sites (Abrol and Sandhu, 1981 and Gill and Abrol, 1986). Some of the tree genera are well known for their tolerance to salt which includes *Acacia, Eucalyptus, Casuarina* and *Prosopis*. Subsequent management of forestry plantations on salt-affected soils including tending, thinning, harvesting and marketing is of little experience todate. However, what is needed to have a system of management which have the advantage to generate adequate income for the farmer/owner through marketing of forestry products with comparatively less maintenance cost and without deteriorating the condition of soil. It seems to be feasible to maintain such plantations through coppice management which is quite suitable for production of firewood and small size timber including poles. This will not only reduce the cost of planting but the area will not be exposed after harvesting.

CHAPTER 3

SALT TOLERANCE IN PLANTS - A REVIEW OF LITERATURE

3.1 INTRODUCTION

Excessive salinity is one of the facts of plant life, and hence of all life, especially in arid and semi-arid parts of the world. The damage caused to plants by salinity can be osmotic, toxic or nutritional and salinity may inhibit growth through disturbances in the water balance and turgor, depletion of energy required for the metabolism involved in growth, or the stress caused by toxicity of particular ion, e.g. Cl^- (Levitt, 1980; Wyn Jones, 1981 and Poljakoff-Mayber, 1982).

Plants adversely effected by salinity grow more slowly and appear stunted (Bernstein, 1975). The increased osmotic pressure of saline soil solution tends to restrict uptake of water by plant roots. All plants are subject to this influence, although sensitivity to this effect varies widely with plant species (Bernstein and Hayward, 1958).

Counteracting these osmotic effects involve excessive accumulation of ions from the external medium or synthesis of other osmotica such as organic and amino acids (Poljakoff-Mayber, 1982), thereby acquiring an internal concentration of osmotically active solutes sufficient to maintain water flow into plants (Rains, 1972 and 1979). The production or absorption of sufficient osmotica is metabolically expensive, potentially limiting the plant by consuming significant quantities of carbon that could otherwise be used for growth (Rains, 1978).

Species respond differently to ionic and osmotic influences of salinity, and genotypes also respond differently to specific ions (Shannon, 1982). The differences in the response of plants to salinity depends on their physiological requirements. Plants in nature have evolved a number of adaptive mechanisms to cope with the presence of salt in their environment and the most significant of these mechanisms entails actual tolerance by plants of high levels of salt within their tissues (Rains *et al.*, 1980).

The ability of a plant to grow and complete its life cycle on saline soils is termed "salt tolerance", which has two requirements: osmotic adaptation and the acquisition of the mineral elements needed for growth and functional metabolism (Jeschke, 1984). Levitt (1980) defined "salt tolerance" as ion accumulation in the absence of negative effects on growth. According to Yeo (1983), the degree to which growth and normal metabolism can be maintained in such non-optimal conditions can be described "resistance to salinity".

Salt tolerance is an important property for plants, which at any time during their life cycle, are exposed to the effects of salts. Such effects might be temporary, e.g when root pass through saline soil layer or permanent.

The osmotic adjustment to an increase in salt in the medium, generally involves a check in expansion growth followed by recovery; this is commonly reported to occur in response to water deficit (Meyer and Boyer, 1981 and Munns and Weir, 1981). The mechanism of osmotic adjustment also varies among plant species (Chapman, 1962) and the genetic variability within a species is also likely to markedly effect tolerance to salt (Blake, 1981).

3.2 PHYSIOLOGY OF SALT TOLERANCE

'Halophytes' are plants which survive high concentrations of electrolytes and are adapted to complete their life cycle in salinities about the same as sea water (171 to 684 mol m^{-3}) (Flowers *et al.*, 1986). These environments are normally dominated by sodium chloride (NaCl) but many contain a variety of other salts like Na_2SO_4, $MgSO_4$, $CaSO_4$, Mg Cl, KCl and Na_2CO_3 (Waisel, 1972 and Flowers, *et al.*, 1977). Plants which generally grow well only under conditions with no salt or only traces of 'salt are known as 'glycophytes' (Levitt, 1980) and the most plants are glycophytes.

Salt tolerance of plants generally means the sustained growth of plants in an environment of NaCl or combination of mixed salts (Shannon, 1984) and it is usually evaluated according to the following three criteria (Hayward and Wadleigh, 1949; Hayward and Bernstein, 1958; Richard, 1969; Shannon, 1984 and Flowers and Yeo, 1989).

(i) Survival of plants under conditions of high concentrations of salts. But it is just possible that the plants might survive at high level of salinity without any or little growth. For example many halophytes can withstand high salinities by such strategies as temporary dormancy, increase succulence or shortening of the growing season. However, dormancy is not compatible with high yields and an increase in succulence contributes nothing to dry weight.

(ii) Another criteria of evaluating salt tolerance is the growth or yield response under saline conditions. This criterion might give more reflection of vigour than actual tolerance. For example the absolute yield of three plants might differ substantially at different salinities.

(iii) A better basis for comparison among diverse crops can be obtained through the relative yield of the crop on a saline soil as compared with its yield on a non-saline soil under similar growing conditions. Alternatively salt tolerance can be measured in terms of relative reduction in yield as a function of increasing soil salinity.

Plants differ widely in salt resistance, from sensitive ones that are prevented from normal growth by even low concentrations of salt, to the most resistant halophytes from saline habitats. Salt tolerant species may also differ in the mechanism by which they withstand high concentrations of salts. Levitt (1980) described two possible kinds of salt resistance under primary stress i.e. avoidance and tolerance (Fig. 3.1).

Through avoidance plants can use any one of three methods to avoid salt stress; (a) exclude the salt passively, (b) extrude it actively and (c) dilute the entering salt.

Salt avoidance is the strategy that a plant uses to escape salinity effects e.g. delayed germination or maturity until more favourable conditions prevails; the exclusion of salts at root zone or compartmentation and secretion by specialized glands or organelles (Shannon, 1982).

Halophytes 'by definition' tolerate high levels of salinity and can also regulate the uptake and distribution of ions within the tissues. Glycophytes, on the other hand, respond to salinity basically by ion exclusion (Flowers *et al.*, 1977). Moderately resistant, non halophytic plants owe their resistance primarily to avoidance e.g. barley (Greenway, 1965). According to Greenway when halophytes and non-halophytes are compared, ion accumulation (salt tolerance) appears to be a superior mechanism for growth in saline environment. This is why the roots of *Atriplex vesicaria* instead of excluding or extruding the salt, mainly function in absorbing it and transporting it to the leaves (Black, 1960). Halophytes demonstrate true salt tolerance, by accumulating salts and, in many instances, by having requirements for it in order to obtain maximum growth (Shannon, 1982).

The adaptive feature of the ion accumulation mechanism is linked to osmotic adjustment and the plants' ability to extract water from the surrounding medium of low water potential. The accumulation of salts and the synthesis of organic substances reduce the osmotic potential differences between plants and soil water and osmotic adjustment in halophytes is generally achieved by means of ion accumulation in the roots (Flowers *et al.*, 1986).

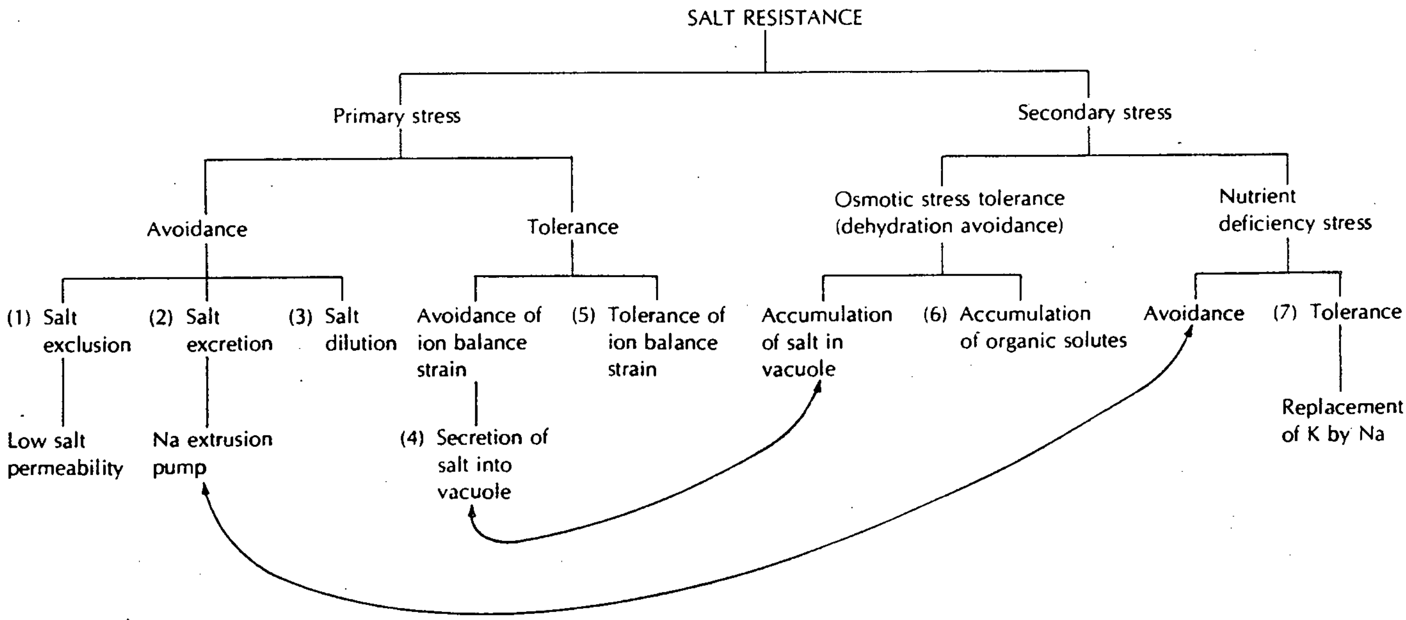

FIGURE 3.1: The possible kinds of salt resistance (Na salt)(Source: Levitt, 1980)

The mechanisms for osmotic adjustments vary among plant species and are the basis for one major classification of halophytes (Bernstein, 1975). Those that accumulate salts in effecting osmotic adjustments are known as euhalophytes; those that accumulate organic solutes rather than salts are called glycohalophytes.

According to Greenway and Munns (1980), salt sensitivity in non-halophytes may result from (i) inability of osmoregulation which is either due to insufficient uptake of salt ions or lack of synthesis of organic solutes being used as osmotica; or (ii) injury caused by inorganic ions which are absorbed by the cell and are not compartmentalized.

Bernstein and Hayward (1958) have related the growth retardation in plants to osmotic effects and specific ion effects. According to them, a limiting layer(s) of cells exists within the plant root which delimits the inner boundary of outer space and these cell transmit water by osmotic processes deeper into the plant tissues while the absorption of certain ions is restricted, thereby building up in the outer layer a concentration which prevents further diffusion of this ions into the plant or even promotes an outward diffusion in to the root medium to re-establish equilibrium. The osmotic properties of the cell layer bounding outer space would then become the limiting factor in transmitting the gradient of water potential of the above ground parts to the root medium.

Gauch and Wadleigh (1945) reported that the progressive decrease in growth of red kidney bean plants with increasing osmotic pressure in the range 1 to 4 bar was directly and primarily related to osmotic pressure when NaCl, Na_2SO_4 and $CaCl_2$ were the salts added to the base nutrient solution. However, they did find a depression in total N-concentration in bean plants when high concentrations of NaCl or $CaCl_2$ were added to the culture solutions.

In both halophytes and non-halophytes absorption of solutes is paramount, and the solutes absorbed are ions from the external solution. It is in this respect that halophytes differ markedly from non-halophytes. Most halophytes absorb sodium from the medium, translocate it into leaves and tolerate the high concentrations of it which build up in the leaves (Black, 1956; Collannder, 1941; Rains and Epstein, 1967 and Scholander *et al.*, 1966).

Non-halophytes accumulate less sodium in the roots and transfer little to the leaves. Most of the sodium absorbed is retained in the roots and lower stem (Bernstein *et al.*, 1956; Jacoby, 1964, 1965; LaHaye and Epstein, 1969; Rains, 1969 and Wallace *et al.*, 1965).

Growth of plants, however, needs to be evaluated in terms of dry weight instead of fresh weight which is usually due to the primary effects of succulence. Stoney and

Jones (1979) reported maximum fresh weight of *Artiplex spongiosa* and *Suaeda monoica* in solution culture containing 100 and 150 mol m^{-3} of NaCl respectively. A closer examination indicated that increases in fresh weight of *A. spongiosa* were primarily due to an increase in succulence, whereas dry weight could not be significantly increased. Furthermore, in spite of an almost 300% increase in fresh weight of *S. monoica* at 500 mol m^{-3} NaCl, it was found that dry weight increased by ≈30% only up to 150 mol m^{-3} NaCl; and at least one-half of the dry weight increase was attributed directly to the accumulation of Na^+ salts. Thus, increasing succulence in plants caused by NaCl, may lead to wrong interpretation of growth when expressed in fresh weight instead of dry weight.

Greenway (1962) while comparing the growth and yield of a relatively salt-tolerant with salt-sensitive variety of barley found that the total dry weight increment and various components of grain yield were lowered by salinity treatments. Also the difference in salt tolerance of *Hordeum vulgare* varieties were related to higher chloride and sodium and/or lower potassium concentrations in the sensitive rather than in the resistant varieties.

From all the above evidence, it is obvious that there is not one mechanism of salt resistance; there are several (Fig. 3.1).

Soil salinity is considered to be the most important adaphic factor limiting not only plant growth but also distribution of plants in their natural habitat. Of the possible strategies for coping with it, only that of ameliorating the soil and water has been extensively applied. It has been suggested that these strategies should be complemented by genetic manipulation of plants to adapt them to saline conditions (Epstein *et al.*, 1980). A highly attractive biotic approach to overcome the salinity, viz. selection and breeding for salt tolerance as suggested by Epstein and Norlyn (1977), seems to be efficient and economical. It is now recognized that genetic variability for salt-tolerance exists within and among species and that this variation can be used to develop crops/plants specially adapted to salt-affected soils. The physiology of salt tolerant plants has been investigated to some extent, but very little is known about the inheritance of tolerance (Tal, 1985). Almost nothing is known about the gene that effects the salt tolerance (Shannon, 1984)

The general approach towards testing heritability of a trait like salt tolerance is given in Falconer (1982). It is necessary either to test for the extent of the character in parents and offspring, or to examine the character in families (i.e., in tree species, collection of seed is made from several parent trees). The latter approach is the most applicable to forestry.

According to Epstein *et al.*, (1980) selection and breeding for resistance to any environmental stress ultimately depends on (a) genetic variability for resistance to stress and (b) exposure of a genetically variable population to the stress. The latter facilitates identification of individuals approaching or possessing the desired genotype.

Exploitation of the natural diversity within a population of plants by selection and then breeding is a method of proven effectiveness (Boyer,1982). Epstein and Norlyn (1977) applied selection pressure to a mixture of barley genotypes by growing a large number of plants in culture solutions salinized with NaCl. Plants capable of tolerating high concentrations of salts were grown to maturity and seeds were collected . These plants were then grown in sandy soils irrigated with sea water and several genotypes were identified which performed satisfactorily in the highly salinized environment. From these results, it is apparent that large scale screening of natural populations is a valid procedure (Rains,1979) because genetic variability in the plant kingdom is important in providing the basis for adaptation of plants to a specific environment (Brown, 1979).

Although work along these lines has been done for agricultural crops very few attempts have been made to take advantage of variation in trees for their tolerance to salinity.

According to Epstein (1983) one of the pre-requisites for improving the salt tolerance of agricultural crop species can be suitable genetic variability within a species. Genotypic variation for salt tolerance in crop species has been reviewed by several workers but almost no information is available about trees. Another pre-requisite for improvement of salt tolerance in plants is a method for screening large number of genotypes (Epstein,1983). This requires diagnostic criteria by which salt tolerant genotypes can be easily identified when segregating a large population (Epstein,1983). The major potential selection criteria for salt resistance are summarized in Table 3.1.

According to Richard (1969) and Shannon (1984) the salt tolerance of a crop is assessed according to three criteria : (1) the ability of the crop to survive on saline soils (2) the yield of the crop on saline soils and (3) the relative yield of the crop on saline soils as compared with its yield on a non-saline soils. These criteria can also be applied to select putative resistant genotypes for their salt tolerance.

TABLE 3.1 : Potential Selection Criteria for Salt Resistance (Source Tal,1985)

Growth	
Germination percentage	Useful only in crops that are
Emergence percentage	equally resistant during
Seedling survival stage	germination and later growth stages
Rooting	
Ions	
Accumulation	Indicates tolerance in salt includers
Exclusion	Indicates tolerance in salt excluders
Membranes	
Leakage of electrolytes	Indicates sensitivity
Abnormal plasmolysis	
Vital staining	May indicate disturbed or undisturbed metabolism
Chlorophyll fluorescence	Changes in its parameters may be used for screening for salt tolerance.

3.3 EFFECT OF SALINITY ON PLANT GROWTH

Saline soils contain soluble salts in quantities that affect adversely the growth of plants. The plants on saline soils grow more slowly (Bernstein, 1975). Salinity inhibits plant growth either through disturbances in the water balance and reduction in turgor or through depletion of energy required for metabolism in the growth, or both. These disturbances might result either from difficulties in uptake of water and transport within the plant or from toxic effects caused by excess of mineral ions in the tissue (Poljakoff-Mayber, 1982). Plants are normally categorized on the basis of their growth response to salt (Greenway and Munns, 1980).

The damage caused by salinity stress can be (i) osmotic (ii) toxic (iii) nutritional (Acevedo *et al.*, 1979). The response of the plant to high salinity depends on number of factors such as plant species, the nature and composition of salinity, the development stage of plant and environmental conditions (Bernstein, 1975).

The effects on plant growth of excessive concentration of soluble salts in the root medium might be specific effects of constituent ion(s) in the saline media, or a combination of the two (Bernstein and Hayward, 1958). According to Poljakoff-Mayber (1982) plants usually respond to a decrease in water potential of the root medium by decreasing their water potential. This is usually considered as an osmotic adaption which permits the maintenance of the potential gradient for uptake of water and of a positive turgor. Maintenance of turgor is an obligatory condition for growth of plants. Any reduction in turgor due to salinity, even if temporary, is liable to inhibit growth (Hoffman *et al.*, 1980) and is explained as being due to;

(a) utilization of photosynthates not for growth but for osmoregulation;
(b) diversion of part of energy derived by respiration to synthesis of the organic osmotica or to the maintenance of the ion uptake mechanism, or to damage repair instead of usual cellular events;
(c) damage to the enzyme proteins exposed to the low water potentials and to the relatively high ionic strength; and,
(d) partial closure of stomata and hence interfering with CO_2 uptake.

(Stepnoikus, 1980)

The decreased growth of plants in saline soils may be caused by suppression of nutrient absorption due to uptake of NaCl in competition with nutrient ions (Levitt, 1980). Thus even when the osmotic stress was eliminated, the growth of *Phaseolus vulgaris, Pisum sativum* and *Citrus aurantium* was decreased by the salt stress (Giorgi *et al.*, 1967).

Solov'ev (1969) concluded that the main cause of NaCl-induced growth inhibition is the difficulty in uptake of mineral nutrients due to competition with Na^+.

According to Kramer (1969) high concentrations of salt cause a decrease in permeability of roots to water and hence decrease in the rate of its entry into the plants. As a result of this hydraulic resistance, entry of water into leaf cells might be so slow as to give rise to a water deficit even though the cells have generated a sufficiently high internal solute concentration to bring about osmotic adjustment (Lagerwerff, 1969; Meiri and Poljakoff-Mayber, 1969; O' Leary, 1969; Riley, 1969 and Epstein, 1972). It is well established that increase in soil salinity also affects plant water relations (Greenway and Munns, 1980) and in order to cope with low osmotic potential, plants absorb ions and transport them to stem and leaves.The accumulation of ions within plant tissues enables the plants to live and survive in the saline soils (Flowers *et al.*, 1977 and Greenway and Munns,1980).

Ion accumulation in the plants mitigate the stress caused by the increase in osmotic pressure of the substrate (Greenway, 1962). According to Greenway and Munns (1980) the poor growth of sensitive varieties at high NaCl is caused by the high ion concentration in leaves. Flowers *et al.*, (1977) distinguished the halophytes from glycophytes by their ability to accumulate ions to high concentrations and particularly in cells of the leaf. However, there are indications that ions differ in their ability to promote growth: for example, Na^+ is more effective than K^+ at low concentrations (Williams, 1960) and K^+ is often inhibiting at high concentrations (Ashby and Beadle, 1957; Baumeister and Schmidt, 1962; Johnson *et al.*, 1968; Keller, 1925 and Williams, 1960)

The growth of *Trifolium alexandrinum* and *T. pratense* grown in solution culture of NaCl, was reduced by 30% and 47% respectively at 50 and 100 mol m^{-3} NaCl, mostly effecting the stem (Winter and Lauchli, 1982). Subsequently Winter (1982) reported that this plant uses several mechanisms to cope with moderate salinity by (i) retranslocation of Na^+ and Cl^- out of young leaves to maintain low concentrations compared with stem and petioles; (ii) predominant basipetal movement of leaf-exported Na^+ and its extrusion into the medium; (iii) establishment of a gradient of K^+: Na^+ ratios along the plant axis providing highest K^+ : Na^+ in youngest stem parts and leaves; and, (iv) restricted transfer of Cl^- from roots to shoots leading to moderate Cl^- content distributed equally in stem tissues of different ages.

Salim and Pitman (1983) reported that older leaves of bean grown at high salinity usually have higher Cl^- concentrations than younger leaves.

Tal *et al., (1979)* while comparing the responses of the wild tomato species to high NaCl levels, relative to those of the cultivated tomato, found that Na^+ and Cl^- were higher in the leaves and shoot apices. Greenway and Munns (1980) in their review on mechanisms of salt tolerance in non-halophytes, reported that more resistant species adopted ion exclusion along with a lesser component of ion compartmentation in the roots. And, the exclusion by compartmentation in the roots was efficient enough to prevent transport of Na^+ and Cl^- to the shoot where classic salt injury symptoms were observed. Moderately affected species showed an ion response by partial root exclusion/compartmentation and there was some transport of Na^+ and Cl^- to the shoot. Lauchli and Stelter (1982) reported that salt tolerance in cotton appears related to accumulation of Na^+ and Cl^- in the shoot and to K/Na selectivity in the root.

Kemp and Cunningham (1981) found that salinity induced a decrease in net photosynthesis which was caused largely by stomatal closure in *Distichlis spicata*. Walker and Downton (1982) measured photosynthesis by infrared gas analyzer in mature leaves of citrus (*Citrus medica*), guava (*Psidium guajava*) and grapevine

(*Vities vinifera*) at various times after the commencement of NaCl treatments. The treatments were in ranges 0-50, 0-75 and 0-90 mol m^{-3} for citrus, guava and grapevine respectively. In all cases, photosynthesis was progressively reduced by salinity.

Most of the studies have concluded that decline in photosynthesis in response to increased salinity is to some extent the result of stomatål conductance (Longstretch and Nobel, 1979 and Robinson *et al.*, 1983). Pezeshki and Chambers (1986) found decrease in stomatal conductance, net photosynthesis and plant water potential in green ash (*Fraxinus pennsylvanica*) in response to application of saline water. Both responses were rapid and occurred shortly after treatment. Walker (1989) found decrease in photosynthetic rate per unit leaf area and chlorophyll at salinities above 100 mol m^{-3} Cl^- accompanying with cation Na^+, Ca^{2+} and Mg^{2+} in *Santalum acumiriatum,* an Australian native peach tree.

Meiri and Poljakoff-Mayber (1970) reported that in all the salinity regimes studied, the growth of bean plants (*Phaseolus vulgaris* var. Brittle Wax) was depressed and it was expressed in terms of lower dry weight yield, smaller number of leaves per plant and smaller area of individual leaf. The smaller leaf area in the treated plants was due to both, smaller leaves and a smaller number of leaves. It was found that the shoots especially leaves were affected more severely by salinity as compared to roots; thus the root/shoot ratio decreased. As salinity increases most plants have fewer leaves and poor shoot development (Larcher, 1975).

Ratner (1945) studied the influence of transpiration on absorption of minerals by plants grown on saline soils. He distinguished between transpiration effects on low-salt and high-salt plants. In the former transpiration rate was relatively ineffective and he concluded that the salt may have been actually absorbed by root cells and translocated independently of the transpiration stream. In high-salt plants, however, transpiration was effective in increasing salt uptake. According to Ratner the high rate of transpiration in plants on saline soil was damaging because of increased salt absorption. It seems likely, however, that high transpiration simply increased the moisture deficit in the plants, thereby impairing growth.

Longstreth *et al.* (1984) found increases in leaf thickness induced by an increase in NaCl concentration from 0 to 400 mol m^{-3} which slightly compensated the negative effects of salinity on leaf cell metabolism. Also the dry weight yield was reduced by salinity as much as 84% in plants grown in 0 - 400 mol m^{-3} NaCl. Total leaf area per plant was also reduced drastically. Robinson *et al.*, (1983) showed that salinity reduced fresh and dry weight of both shoots and roots to less than 50% of that of control Spinach plants. The salt-affected plants had much thicker leaves and osmotically adjusted to maintain leaf turgor and K^+ in leaf was decreased whereas Na^+ and Cl^- were greatly increased while leaf area decreased.

Increasing NaCl concentration in the rooting medium significantly reduced the total chlorophyll, leaf water potential, leaf solute potential and leaf turgor in black gram (*Vigna mungo L.*) (Ashraf, 1989).

3.4 EFFECTS OF SODIUM AND CHLORIDE ON GROWTH

Sodium chloride is the most commonly considered source of salinity and in general it is well documented that it has adverse effects on plant growth of most species (Eaton, 1942; Greenway, 1962 and Winter and Lauchli, 1982).

Sodium is not known to be generally required by green plants. However, certain halophytes not only tolerate the high concentrations of salt but actually required sodium. *Atriplex vesicaria* is a perennial pasture species which requires sodium as a micro-nutrient element (Brownwell and Wood, 1957 and Brownwell, 1968).

Williams (1960) observed that *Halogeton glomeratus* (M. Bieb) absorbed large quantities of Na and according to him it was essential for vigorous growth of *Halogeton* . Sodium is also known to be essential for *Anabaena cylindrica* Lemm, a blue green alga (Allen and Arnon, 1955).

The work of Brownwell (1968) on twenty three species of higher plants for their response to Na_2SO_4 showed that a species of *Atriplex* developed characteristic sodium-deficiency symptoms and their dry weight yield increased significantly on receiving sodium. Also significant dry weight response to sodium was obtained in *Hordeum vulgare.*

For *Suaeda maritima* Yeo (1974) reported that there was a linear relationship between the dry weight of *Suaeda maritima* plants and the total concentration of ions in the solution for both dilution of the culture solution and addition of NaCl.

The ions differ in their ability to promote growth; for example, Na^+ is more effective than K^+ at low concentrations (Williams, 1960). Glenn and O'Leary (1984) reported that the growth of some halophytes was stimulated by 180 mol m^{-3} NaCl. Previously, Yeo and Flowers (1980) reported stimulation of growth in *Suaeda maritima* by NaCl and it was attributed to the relationship between uptake of salt and turgor pressure leading to enhanced extension growth.

Waisel (1972) reported that growth of *Atriplex hamila* was stimulated by addition of smaller quantities of NaCl to the medium. Harmer *et al.*, (1953) concluded that the positive effects of Na^+ on growth of plants can frequently be seen more clearly in species with higher requirements for potassium.

For *Atriplex vesicaria* the addition of 0.6 mol m^{-3} NaCl to Hoagland and Arnon solution (20 mol m^{-3}) doubled the dry weight, producing some 87% of the maximum effects of 20 mol m^{-3} NaCl on the growth (Black, 1960). The growth of *Atriplex nummularia* was promoted to 108% by addition of 10 mol m^{-3} NaCl to a culture solution of that in the 1 mol m^{-3} NaCl (Greenway, 1968).

Sodium chloride was considered essential for *Salicornia olivieri* at flowering stage (Waisel, 1972). Jennings (1976) reported that small amount of chloride improved growth in higher plants.

According to Sutclife and Baker (1974) chloride has an important role in the production of oxygen and acts as an electron transporting agent in photophosphorylation. Also Eaton (1942) found significant increase in yield of tomatoes and cotton. Jennings (1968) reviewed the physiology of ionic relation of halophytic higher plants and concluded that the effect of sodium on succulence is likely to be a general phenomenon, with mesophytes as well as xerophytes and halophytes. Gale and Poljakoff-Mayber (1970) found very significant stimulation of growth of salt bush (*Atriplex halimus L.*) by low concentrations of NaCl or Na_2SO_4.

The essentiality of chloride was also reported by Broyer *et al.*, (1954) in an experiment with tomato plants grown in purified nutrients solution. The chloride ion was considered to be important in inducing succulence (Bannister, 1967 and van Eijik, 1939). Under chlorine salinity an increase in thickness of leaves is often observed (Hayward and Long, 1941 and Lagerwerff and Eagle, 1961). Meiri and Poljakoff-Mayber (1967) reported increase in leaf thickness of bean plants (*Phaseolus vulgaris*) when sodium chloride salinity was induced in the growth medium. Chloride ions for most species are readily absorbable, so it generally contributes much more to osmotic adjustment as compared to sulphate (Bernstein, 1975). Ulrich and Ohki (1956) reported that chlorine was necessary for top and root growth of sugar beet (*Beta vulgaris*) and was associated with sugar formation rather than with sugar utilization. With potassium in range 0.5-12 m-equiv./L, addition of as little as 0.5 mol m^{-3} sodium chloride could increase both the fresh and dry weight of sugar beet plants. The evidence available showed that part of the increase could be ascribed to chloride ions and part to sodium ions.

3.5 RESEACH ON FOREST TREES

In spite of the realization of the importance of providing a protective vegetative cover on salt- affected areas, the effects of varied salt concentrations on the germination of seeds and the growth of seedlings of different tree species has not been sufficiently investigated. Several tree species have been identified to be considered suitable for planting on salt affected soil in different parts of the world but their relative growth behaviour under different concentrations of salt still needs to be assessed.

Among them the physiological aspect of salt tolerance in some of the tree genera e.g. *Eucalyptus*, *Casuarina* and *Acacia* has been studied to some extent by different workers but very little is known about their inheritance for salt tolerance. Also almost nothing is known about the variability for salt tolerance within the populations of the species.

Eucalyptus species are widely recognized as useful trees for their tolerance to many environmental stresses, including salinity. Attempts have been made in the field as well as under controlled conditions to determine the relative salt-tolerance of *Eucalyptus* species. Blake (1981) determined the relative tolerance to salt of 52 *Eucalyptus species* by applying a liquid culture technique and found marked differences between the species and 11 out of 52 species and sub-species exhibited a high degree of salt tolerance. He suggested that genetic differences within a species are likely to markedly affect the salt tolerance.

Sands (1981) also reported differences in salt tolerance between different provenances of *E. camaldulensis* and proposed a comprehensive screening programme within that species. Omran (1986) tested nine Australian *Eucalyptus* species and provenances and a local seed source of *E. camaldulensis* (Alexandria, Egypt) and reported differences for salt tolerance not only within species but within the provenances of *E. camadulensis* . After two growing seasons, *E. occidentalis* was found to be most tolerant species to salinity followed by *E. camadulensis* (West Wiluna, Australia) and *E. microtheca.*

Among earlier attempts Karschon and Zohar (1975) studied the effects of flooding and salinity on *E. camaldulensis* from three seed sources. They reported significant differences in ability of salt tolerances related to origin of seed. Hart (1972) has considered *E. camaldulensis* suitable for reclamation of moderately saline areas.

Beside *E. camaldulensis,* several other species like *E. tereticornis* and *E. microtheca* are also reported to be salt tolerant to some extent (Malik and Sheikh, 1983; Blake, 1981 and Omran, 1986).

Moezel *et al.*,(1988) tested the response of six *Eucalyptus species* and *Casuarina obesa* to the combined effect of salinity and waterlogging. Seedlings of *E. camaldulensis, E. comital-vallis, E.kondininensis, E. lesouefii, E. platycorys, E. spathulata and Casuarina obesa* were grown in a glasshouse under non-saline drained, saline waterlogged conditions. They found that *C. obesa* was the species most tolerant of saline watrerlogged conditions and lower reduction of growth compared to the *Eucalyptus species*.

Moezel *et al.*, (1989) assessed the tolerance to salinity of five *Casuarina species* by survival and relative growth. They reported that salinity tolerance of *C. obesa, C. glauca and C. equisetifolia* was associated with exclusion of Na^+ and Cl^- while relatively sensitive species, *C. cunninghamiana* and *C. cristata* accumulated salts in the shoots. Clemens *et al.* (1983) reported a wide range of response to salinity in 11 *Casuarina species* with some (e.g. *C. equisetifolia*) showing little growth reduction and no visible injury symptoms.

Hansen and Munns (1988) studied the effects and interactions of varying levels of $CaSO_4$ and NaCl on the growth and nitrogen fixation of *Leucaena leucocephala* . While NaCl significantly reduced plant growth, addition of $CaSO_4$ increased plant height, leaf number and biomass of salt-treated plants. The promotion of *Leucaena* salinity tolerance by addition of $CaSO_4$ was attributed to the effect of Calcium in maintaining the selection permeability of membrane.

Among *Acacias, Acacia nilotica* is well known for its resistance to salinity (Bangash, 1977; Khan and Yadav, 1962; Sandhu, 1988; Yadav and Singh, 1970 and Yadav, 1977). Except for a few field studies, very little information is available about its tolerance to varying degree of salinity and almost nothing is known about the presence of genetic variability within this species. In addition to it there are many other important commercial tree species which still need to be investigated in order to determine their relative tolerance to salt and the existence of genetic variation within the species.

CHAPTER 4

EFFECT OF SALINITY ON THE GROWTH PERFORMANCE OF *Sinapis alba* (*L.*) (White Mustard)

4.1 INTRODUCTION

The aim of the present work was to develop a screening programme to identify and produce salt tolerant genotypes that could grow vigorously under high salt stress. In the developing stage of the project it was decided to work with Mustard, as this species is widely available and grows fast. The work on Mustard is to be followed by further studies on forest trees .

4.2 MATERIALS AND METHODS

4.2.1 Preliminary Study

A preliminary study was conducted to determine the concentration of salinity achieved in the media with each application of NaCl solution of different concentrations. For this purpose small plastic pots (16 × 14 cm; 2 litres) were filled with a 1:1 mixture of perlite and vermiculite and were irrigated daily with NaCl mixed in Hoagland nutrient solution . Six different concentration of NaCl (34, 68, 102, 138, 171 and 205 mol m^{-3}) were selected for this part of the study. The experiment was completely randomized with three replications and six treatments.

Media and leachate samples were collected after every 4 hours and 24 hours of the application of saline solution. The saline solution was added to the media in sufficient quantity to keep it fully saturated all the time.

The electrical conductivity of the leachate and the extract of a mixture of the media and deionized water (1:5) as recommended by Richard (1969) and Landon (1984) was determined with Water Analyzer (PWA-1, Jencons Scientific Ltd., Leighton Buzzard, Beds, U.K) by using the conductivity sensor with a cell constant K=1.09 at 25 °C.

The results obtained for the electrical conductivity of the media extract could not be related to the amount of salt adsorbed by the media because it was not possible to prepare a paste of the latter to obtain an extract in the prescribed manner (Richard, 1969) for measurement of electrical conductivity. Therefore, it was considered appropriate to use the electrical conductivity of the leachate to determine the concentration of salt in the medium.

The data recorded regarding the electrical conductivity of leachate collected every 24-hours after the application of saline solution indicated that a steady state of electrical conductivity is reached after six irrigations, when the salinity of the leachate is equal to that in the irrigation solution (Fig. 4. 1).

4.2.2. Screening Test

Seeds of *Sinapis alba* (White Mustard) were sown in plastic seed trays filled with a mixture of perlite and vermiculite in equal volume (1:1) on 1st August,1989 and they started germinating after 3-4 days . When germination was completed the seedlings were irrigated with weak Hoagland nutrient solution (1/10th), the concentration of which was gradually increased. The experiment was set up in the glasshouse.

The design of the experiment for screening resistant putative genotypes within the population was a randomized block design with seven blocks (replications). The treatments within each block were assigned randomly.

Before starting to apply treatments, the number of seedlings in each tray was recorded. Four levels of NaCl i.e. 34, 102, 205 and 410 mol m^{-3} were selected to contain the range of salinity in the soils of Pakistan (1.3.4). The treatments were started on 14th August,1989 when the seedlings were ten days old.The trays were raised about 3 cm above the ground to facilitate drainage of any excess water or saline solution and to avoid the chances of any root contamination. The seedlings were irrigated daily to maintain the desired concentration of salt in the media. It is assumed that the required level of salinity in the medium was achieved after six applications of saline solution and that thereafter a steady state was maintained.

After eight days (22nd August, 1989) the number of seedlings surviving in each tray was recorded and survival percentages were calculated. Confidence limits were calculated as suggested by Bailey (1959) (Fig. 4.2). The individuals which survived at each level of salinity were considered to be putative resistant genotypes.

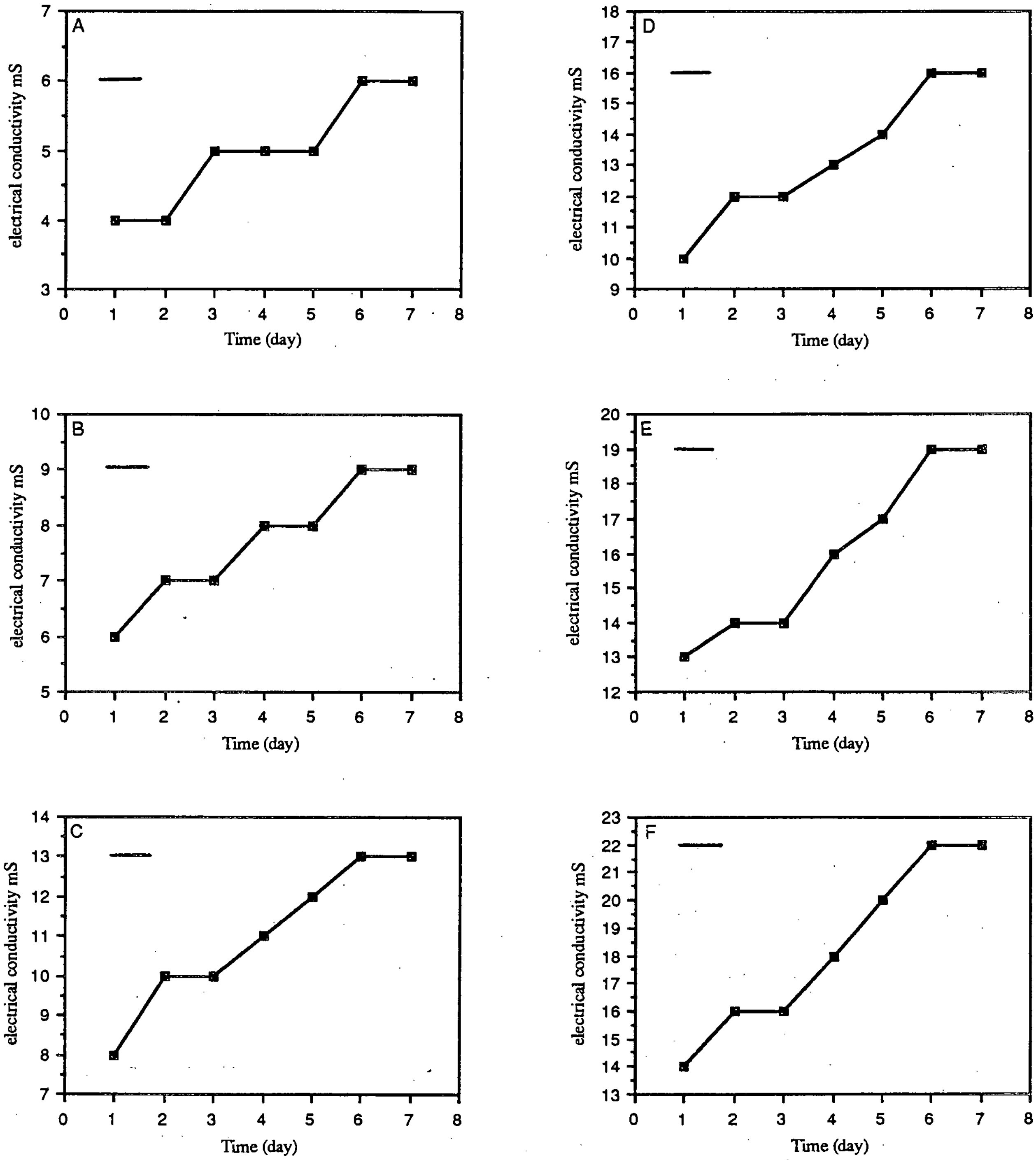

FIGURE 4.1: Electrical conductivity in milli Siemens (mS) of leachate collected after 24 hours of application of NaCl of 34 mol m^{-3} (A), 68 mol m^{-3} (B), 102 mol m^{-3} (C), 138 mol m^{-3} (D) 171 mol m^{-3} (E) and 205 mol m^{-3} (F) mixed in Hoagland nutrient solution. The solution was applied once in a day. The horizontal bar denotes the electrical conductivity of the solution applied.

4.2.3. Growth Trials

For this part of the study 20 uniformly sized seedlings were selected from the resistant genotypes at each level of salinity and transplanted from the trays to plastic pots (height = 21 cm & diameter = 7 cm) perforated at bottom and filled with the mixture of perlite and vermiculite in equal volume (1:1). The experiment was set up in the glasshouse.

The design of the experiment was a randomized block design with ten blocks (replications) and four treatments- three levels of NaCl (i.e. 34, 102 and 205 mol m^{-3}) plus a control.The treatments were assigned randomly within the blocks. Plants treated with one level of salinity during the screening test were assigned to the same level of salinity for growth analysis .

In total 40 seedlings (10 for each treatment) were taken ahead for growth analysis, in addition to 40 seedlings harvested for taking initial growth data.

Before application of treatments, the newly transplanted seedlings were allowed to recover from transplant shock for three days. These were irrigated with tap water for the first day and then Hoagland nutrient solution.

The application of saline solution was started on 27th August,1989 and continued up to 6th September,1989 (10 days). The plants were irrigated with saline solution daily in an amount sufficient to ensure that all water loss was replaced i.e. the media were fully saturated at all the times.

After ten days, the plants were harvested and the leaf, root and stem were separated for each plant. Leaf area was determined by an Area Meter (Model LI-3100, Li-Cor, Lincoln, USA). The plants were oven dried at 70 °C for 7-10 days after which weight measurements of root, stem and leaf were taken.

The average maximum and minimum temperature during the course of the experiment in the glasshouse was 22 °C and 7 °C respectively, and the average maximum and minimum relative humidity was 81% and 60% respectively.

The mean relative growth rate ($\overline{RGR}$), net assimilation rate ($\overline{NAR}$), leaf area ratio ($\overline{LAR}$), specific leaf area ($\overline{SLA}$) and leaf weight ratio ($\overline{LWR}$) were calculated by pairing technique and using the following equations; (Evans,1972 and Hunt, 1978,1982):

$$\overline{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \qquad \text{g g}^{-1}\text{ wk}^{-1}$$

$$\overline{NAR} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e LA_2 - \log_e LA_1}{LA_2 - LA_1} \qquad \text{g cm}^{-2}\text{ wk}^{-1}$$

$$\overline{LAR} = \frac{LA_1/W_1 + LA_2/W_2}{2} \qquad cm^2\ g^{-1}$$

$$\overline{SLA} = \frac{LA_1/LW_1 + LA_2/LW_2}{2} \qquad cm^2\ g^{-1}$$

$$\overline{LWR} = \frac{LW_1/W_1 + LW_2/W_2}{2} \qquad \text{dimensionless.}$$

Where W_1 = total initial dry weight of plant
W_2 = total final dry weight of plant
LA_1 = initial leaf area
LA_2 = final leaf area
LW_1 = initial leaf weight
LW_2 = final leaf weight
t_1 = initial time
t_2 = final time

and the bar denotes that we refer to means over the time interval.

The data obtained were subjected to analysis of variance (using a MINITAB programme) and the standard errors of means were calculated.

4.3 RESULTS

4.3.1 Effect of Salinity on Survival of Seedlings During Screening Test:

Survival decreased with salt concentration in excess of 34 mol m^{-3}, and all plants died at 410 mol m^{-3} (Fig. 4.2).

4.3.2 Effect of Salinity on the Morphology of Seedlings

Symptoms of salt injury started becoming evident as the applications of NaCl progressed. The first effect was the turning of leaves from green to pale and then yellowish. This effect was found at all except the lowest salinity level (34 mol m^{-3}) where there was apparently little effect of salinity on the plants. Indeed, the leaves of

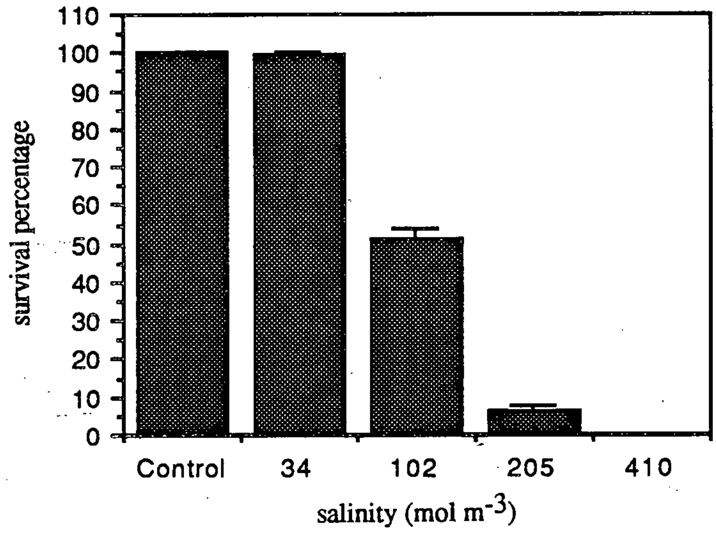

FIGURE 4.2: Survival percentage of *Sinapis alba* grown under different salinity regimes. The vertical bar indicates confidence limits ($P< 0.05$)

plants irrigated with 34 mol m^{-3} became dark green and the seedlings seems to be vigorous and healthy.

Severe leaf burn was observed in some of the plants under higher levels of salinity (102, 205 and 410 mol m^{-3}).

After eight applications the leaves and stems of seedlings treated with the highest salinity level (410 mol m^{-3}) were wilted and they ultimately died.There was no survival at this level of salinity.

4.3.3 Effect of Salinity on the Relative Growth Rate (RGR)

The relative growth rate of the plants decreased significantly ($p < 0.05$) with increase in salinity as compared to the control (Fig. 4.3.A). Maximum depression in relative growth rate was at the highest level of salinity (205 mol m^{-3}) where the RGR is 58% less than the control. The RGR at the lowest level of salinity (34 mol m^{-3}) was only 16% less than the control (Fig. 4.3.A).

4.3.4 Effect of Salinity on Net Assimilation Rate (NAR)

The net assimilation rate was not significantly ($P < 0.05$) effected by salinity (Fig.4.3.B) as compared with control. There was no significant difference between the treatments. This means that NAR was not responsible for the decrease in relative growth rate.

4.3.5 Effect of Salinity on Leaf Area Ratio (LAR)

Leaf area ratio decreased significantly ($P < 0.05$) with increase in salinity with the exception that there was little difference in LAR between plants treated with the lowest level of salinity (34 mol m^{-3}) and control (Fig.4.3.C). The lowest level of LAR was noted in plants under the highest NaCl concentration (410 mol m^{-3})(Fig. 4.3.C).

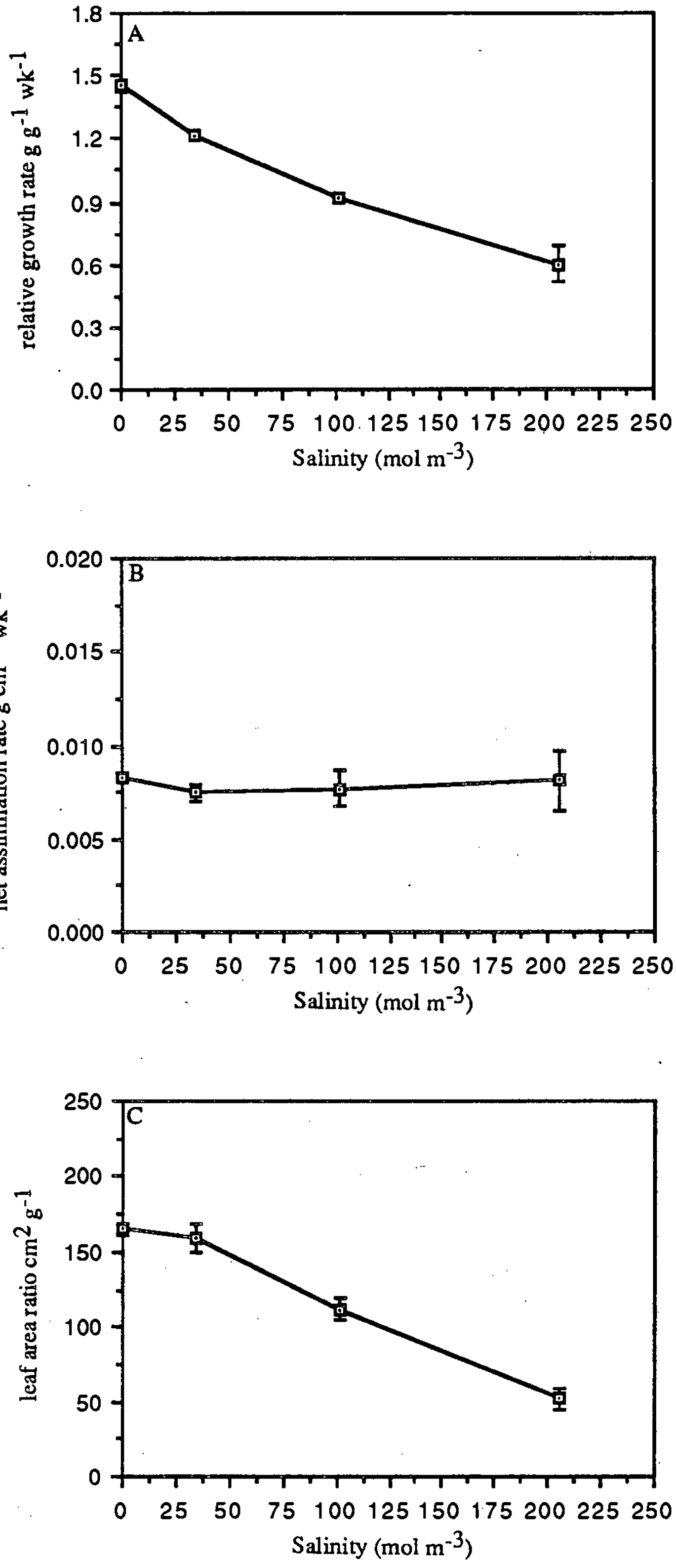

FIGURE 4.3 : Relative growth rate, net assimilation rate and leaf area ratio of *Sinapis alba* grown under different salinity regimes. The vertical bar indicates standard error of mean.

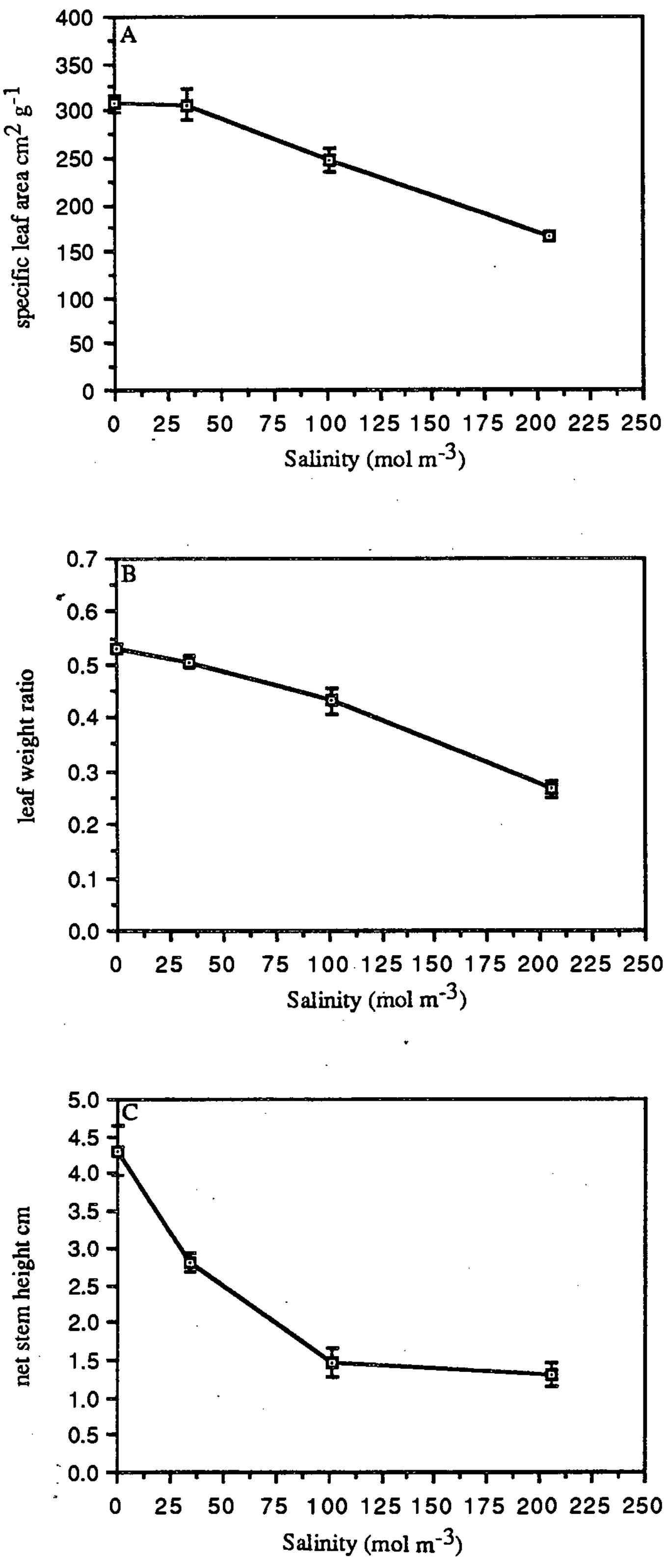

FIGURE 4.4 : Specific leaf area, leaf weight ratio and height of stem of *Sinapis alba* grown under different salinity regimes. The vertical bar indicates standard error of mean.

4.3.6 Effect of Salinity on Specific Leaf Area (SLA) and Leaf Weight Ratio (LWR)

The leaf area ratio is by definition the product SLA × LWR, and so it is possible to see whether the decline in LAR can be attributed to a decline in the area of leaf per gram of leaf, or a decline in the fraction of the entire plant which is leaf.

Specific leaf area was reduced significantly ($P < 0.05$) with increase in salinity (Fig.4.4.A) but there was no significant difference in plants treated with the lowest level of salinity (34 mol m^{-3}) and the control (Fig. 4.4.A).

Leaf weight ratio showed a similar response and the trend of decrease in both SLA and LWR was more or less similar to LAR (Fig. 4.4.B).

4.3.7 Effect of Salinity on Height of Stem

The seedlings of Mustard suffered a large reduction in height of stem with increasing level of salinity (Fig. 4.4.C). The stem height was 69% less than the control under the highest level of salinity (205 mol m^{-3}) whereas it was only 35% less in plants under the lowest salinity (34 mol m^{-3}). However, there was little difference in height of plants treated with the two higher levels of salinity (i.e. 102 & 205 mol m^{-3}).

4.4 DISCUSSION

The results of the experiment show a clear response to NaCl salinity in terms of survival percentage and other growth parameters, are in agreement with others results (Ansari, 1972; Longstreth *et al.*, 1984; Farooq *et al.*, 1989; Walker, 1989; Meiri and Poljakoff-Mayber, 1970; Winter and Lauchli, 1982; Clemens *et al.*, 1983; Curtis and Lauchli, 1985, 1986 and Maas and Hoffman, 1977)

The survival percentage decreased significantly with increase in salinity (Fig. 4.2). However, there was no significant difference in survival at the lowest level of salinity (34 mol m^{-3}) and control and all the plants died at the highest salinity(410 mol m^{-3}). This implies that the species is not tolerant to such a high level of salinity but can survive at lower levels. The presence of survival at 205 mol m^{-3} suggests the existence of genetically controlled salt tolerance within the population, which could possibly be exploited. For example, Epstein and Norlyn (1977) were able to select salt resistant

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genotypes in barley by applying selection procedure and these were allowed to grow until maturity. The plants raised from the seed collected from the resistant genotypes performed well in a salinized environment.

One of the marked effects of salinity was a reduction in leaf area ratio (LAR)(Fig.4.3.C) which in a broad sense represents the ratio of photosynthesizing (Leaf area) to respiring material (total dry matter) within the plant (Hunt, 1978).

In consequence to the decrease in LAR, the relative growth rate (RGR) was significantly affected ($P < 0.05$), decreasing with increase in salinity. The decline in RGR was through a decrease in LAR and not because of a change in net assimilation rate (NAR). In other words it was the reduced leaf area per unit dry material (LAR) which resulted in the decrease in RGR and not the efficiency of leaves as producer of new materials (NAR).

Salinity effects on LAR reflected the salt sensitivity of total leaf area development in *Sinapis alba* showing a significant reduction at 102 and 205 mol m^{-3}. The decrease in LAR with increase in salinity was due to its effects on both specific leaf area (SLA) and leaf weight ratio (LWR). The specific leaf area (allocation of dry material per unit leaf area) and leaf weight ratio (proportion of dry matter allocated to leaf tissue) also decreased at 102 and 205 mol m^{-3} whereas there was no difference between control and the lowest salinity (34 mol m^{-3}).

The early effect of salinity on non-halophytes is that leaves grow more slowly (Munns and Termaat, 1986) because they experience both a decline in water potential and increased tissue ion contents (Curtis and Lauchli, 1986). Salt concentration in individual leaves of non-halophytes usually increases greatly with time (e.g. barley, Greenway, 1962; Greenway and Thomas, 1965 and Walker, Torokfalvy and Downton, 1982) and there is no sign of regulation of the salt concentration in the leaves (Flowers and Yeo, 1986). Thus for non-halophytes it is inevitable that ion concentration is eventually built up in the leaves.

At high salinity the accumulation of ions is considered to be toxic to plants and to adversely effect their growth (Morris, 1980) the degree of salt injury being related to the concentration of Na^+ and/or Cl^- ions within the plants (Greenway, 1962). The rate of accumulation of Na^+ and Cl^- in the leaves mainly depends on the ability of roots to exclude salt from the transpiration stream and on the volume flux of the transpiration stream (Munns and Termaat, 1986)

The accumulation of ions in plant tissues results in a reduction in cell elongation and division (Waisel, 1972), a consequent decline in leaf area to such an extent that the plant can no longer produce enough carbohydrates to support continued growth.

Previous studies conducted on the effect of salinity on non-halophytes showed a similar response in terms of depression in growth and decline in leaf area. Meiri and Poljakoff-Mayber (1967) reported that sodium chloride salinity induced retardation of leaf growth in bean plants (*Phaseolus vulgaris*) and that the growth in area ceased before growth in leaf thickness. The reduction in growth of leaves was the result of reduction in cell size. Seemann and Critchley (1985) found a decrease in dry and fresh weight and leaf area in *Phaseolus vulgaris* and reported that the growth reduction was likely to be a consequence of a number of differing effects of salts on plant processes, including effects on photosynthetic performance.

Curtis and Lauchli (1986) found a significant decline in LAR in Kenaf (*Hibiscus cannabinus*) and no decrease in net assimilation rate. They concluded that growth in Kenaf under moderate salt stress was affected primarily through reduction in expansive growth and leaf area development rather than any decline in photosynthetic capacity. Nerson and Paris (1984) reported that salinity affected the leaves more than the stem in melons (*Cucumis melo*) and leaf area was more sharply reduced than stem length. Curtis and Lauchli (1985) found a linear decline in rate of leaf emergence and leaf relative growth rate with increasing salt stress.

The data suggest a decrease in specific leaf area (SLA) which implies that the leaves of *Sinapis alba* got thicker than the control under 102 and 205 mol m^{-3}. One of the widely reported response to salinity is an increase in leaf thickness (Jennings,1976; Longstreth and Nobel,1979; Poljakoff-Mayber,1975 and Waisel,1972). Longstreth *et al.*,(1984) found an increase in leaf thickness and decrease in area per unit dry weight (LAR) of *Alternanthera philoxeroides* (Mart) Griseb. Kemp and Cunningham (1981) reported an increase in leaf thickness of *Distichlis spicata* with increase in salinity.

In conclusion, the work described in this chapter has shown how putative resistant genotypes can be obtained by growing plants in a salinized medium. It has also shown how classical growth analysis can be used to provide an insight into the way salt affects the growth of the young plants after they have been transplanted. In particular, the *Sinapis* work suggests that assimilation per unit of leaf area is not affected by salt but the area of leaf per weight of plant is affected. In the next chapter, similar techniques will be applied to *Eucalyptus*, but growth rate of selected versus non-selected populations will be compared in a more direct way.

CHAPTER 5

EFFECT OF SALINITY ON THE GROWTH OF *Eucalyptus microtheca* F.Muel.

5.1 INTRODUCTION

The object of this experiment was to (a) assess the genetic variation for salt resistance within a population of *Eucalyptus microtheca* by identifying putative resistant genotypes and (b) evaluate the subsequent performance of the latter by measuring growth at different levels of salinity .The methodology developed with Mustard (chapter-4) was applied.

5.2 MATERIALS AND METHODS

Seed of *Eucalyptus microtheca* was obtained from Pakistan (it is not clear whether the seed was collected from one or more sources) and the experiment was set up in a glass house.

On the basis of a germination test , 0.4 grams of seed was sown in plastic trays filled with perlite and vermiculite mixed in equal volumes on 16th October,1989. The seed started germinating after ten days. After completion of germination, Hoagland nutrient solution (1/10th strength) was applied daily, the concentration being progressively increased to full strength over a period of 30-35 days.

5.2.1 Screening Test

In order to eliminate salt- intolerant individuals 3 levels of NaCl solution plus a control of just Hoagland nutrient solution were applied. The design of the experiment was a randomized block design with six blocks (replications) and four treatments (Control, 102 mol m^{-3}, 205 mol m^{-3} and 410 mol m^{-3} NaCl). Treatments were assigned randomly within blocks. The trays were raised about 3 cm above the surface of the bench to avoid any chances of contamination of roots and allow quick drainage of any excess water. Before application of treatments the total number of seedlings in each tray was recorded.

The treatments were started on 4th December,1989 when the seedlings were 30-35 days old. The seedlings were irrigated daily with the three Nacl concentrations mixed in Hoagland nutrient solution to achieve and maintain the required level of salinity in the growth medium. It was assumed that this was achieved after 6 days and that thereafter a steady state was reached, as suggested by the results of the pilot study (Fig.4.1). The saline solution was added every time in amount sufficient to ensure that all solution in the medium was replaced and the growing medium was at or close to field capacity all the time.

Treatment effects in the form of yellowing of leaves, wilting and dying of some of the seedlings became evident from 11th December, 1989 (after 8 applications of saline solution). The application of saline solution continued until 23rd December, 1989 (treatment period = 20 days) when differences in treatment effects were clear and survival was recorded. The plants which survived at each level of salinity were considered to be putative resistant genotypes with respect to salt tolerance. These individuals were taken forward for further investigations in the second stage of the experiment.

Daily maximum and minimum temperature and relative humidity were recorded by a Thermohygrograph (Cassella, London, U.K., Fig. 5.2)

5.2.2 Growth Trials

Uniform sized plants surviving from the screening test were transplanted from trays into plastic pots (23.5 cm high × 8 cm diameter) perforated at the bottom to allow drainage and filled with a mixture of perlite and vermiculite in equal volumes. At that stage the seedlings were about 6-18 cm tall. All plants were first irrigated with tap water for one or two days and then with full strength Hoagland nutrient solution (irrespective of the source treatment) and were allowed to grow until 9th January,1990 so that they should fully recover from any transplant shock.

The design of the experiment was a randomized block design with seven blocks (replications) and four treatments- three levels of NaCl solution (102 mol m^{-3}, 205 mol m^{-3} and 410 mol m^{-3}) plus a control (the same treatments as in the screening test). For this part of the study (unlike the Mustard experiment), all possible combinations of the four current treatments and four source treatments were made giving the following sixteen treatment combinations :

Source		Treatment assigned
Control	+	Control
Control	+	102 mol m^{-3}
Control	+	205 mol m^{-3}
Control	+	410 mol m^{-3}
102 mol m^{-3}	+	Control
102 mol m^{-3}	+	102 mol m^{-3}
102 mol m^{-3}	+	205 mol m^{-3}
102 mol m^{-3}	+	410 mol m^{-3}
205 mol m^{-3}	+	Control
205 mol m^{-3}	+	102 mol m^{-3}
205 mol m^{-3}	+	205 mol m^{-3}
205 mol m^{-3}	+	410 mol m^{-3}
410 mol m^{-3}	+	Control
410 mol m^{-3}	+	102 mol m^{-3}
410 mol m^{-3}	+	205 mol m^{-3}
410 mol m^{-3}	+	410 mol m^{-3}

A total of 112 seedlings were used (one for each treatment combination × 7-blocks) in addition to 28 seedlings (7 from each source treatment) that were sacrificed at the begining to obtain initial weights and leaf areas.

The seedlings (in pots) were placed in plastic trays which were raised 3 cm above the surface of the experimental bench in order to avoid contamination of roots and provide easy and quick drainage of any excess water or saline solution.When assigning plants to blocks, the blocking pattern of the screening test (5.2.1) was maintained while treatments within blocks were allotted randomly . The set up of the experiment on the 2.75 m × 1.83 m bench is illustrated in plate-1.

The application of salinized solution was started on 9th January,1990. The plants were irrigated daily with salinized solution in amounts sufficient to ensure that all old solution was replaced by fresh solution and the medium was maintained at or close to field capacity.

The solution that leached from the pots (3 replicates for each treatment combination) was collected, first after one week and then after ten days. The electrical conductivity of this leachate was measured by a Water Analyser (PWA-1, Jencons Scientific Ltd., Leighton Buzzard, Beds, U.K.) using the conductivity sensor with a cell constant K = 1.09 at 25 °C. After 6-7 applications of saline solution, the electrical conductivity of the leachate was the same as that of the saline solution applied,

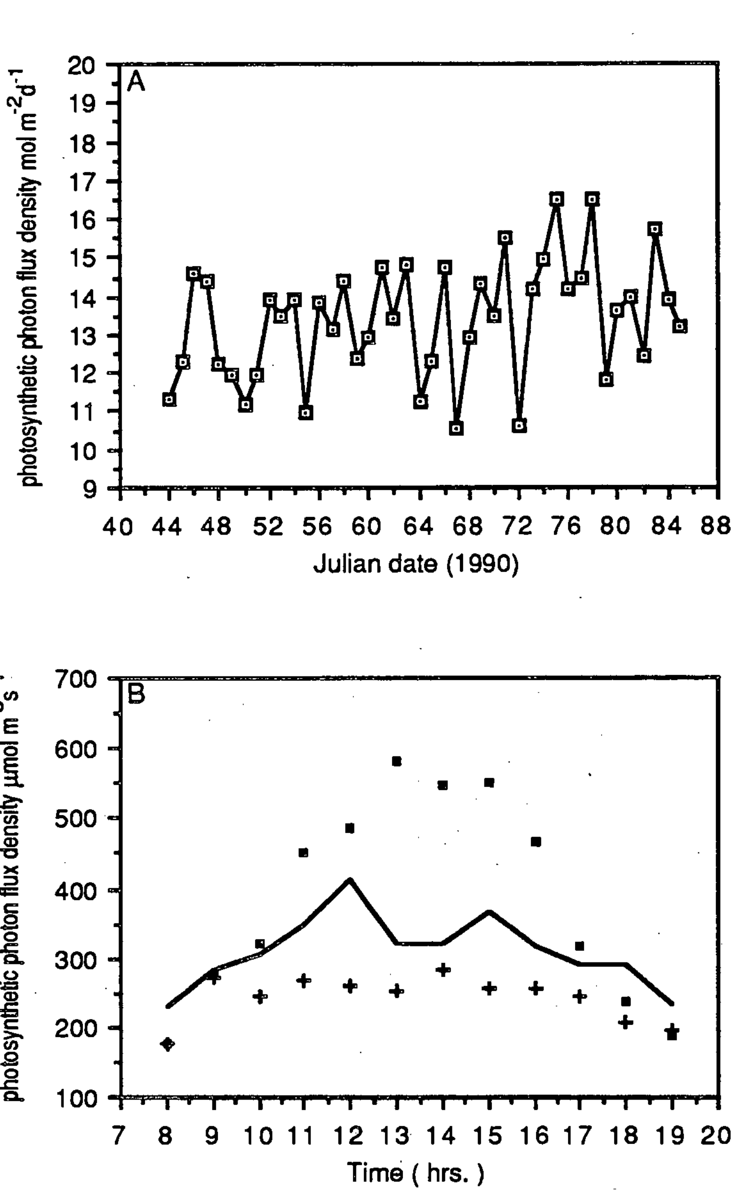

FIGURE 5.1: Light environment on the experiment bench. (A): Total daily photosynthetic photon flux density over time; (B): photosynthetic photon flux desity over a day of medium irradiance(3rd March)(–), high irradiance(16th March)(▪)and low irradiance(8th March,1990)(+).

Plate 1: Growth trials of *Eucalyptus microtheca* set-up in the glasshouse as a randomized block design illuminated with Mercury Vapour Fluorescence lamps.

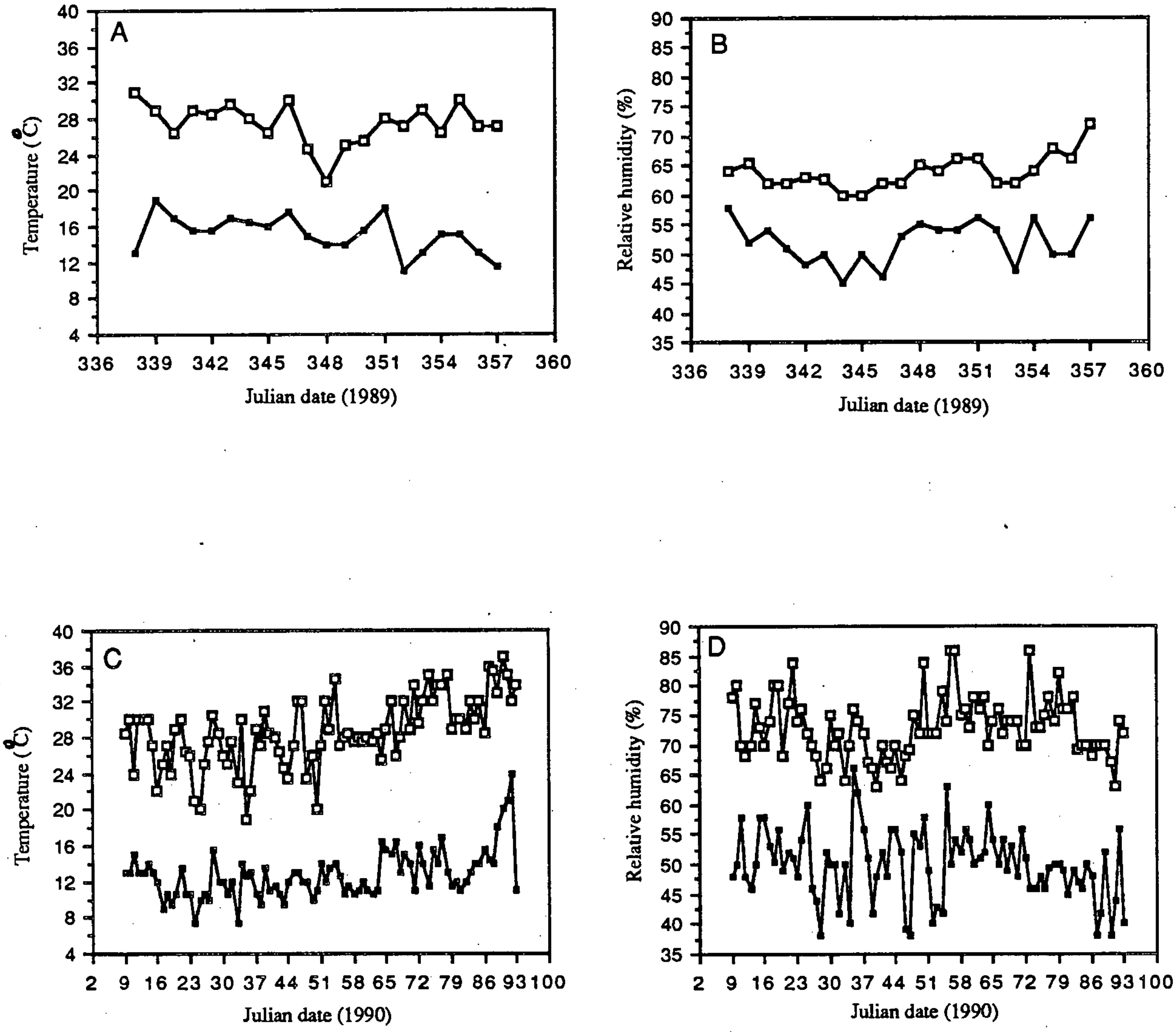

FIGURE 5.2. Temperature and relative humidity during the experiment. A-B during screening test and C-D during growth trails. (-□-) and (-■-) indicates maximum and minimum values respectively.

thereafter staying constant. Hence we can assume that the desired level of salinity in the growth medium was achieved.

The experimental bench was illuminated with Mercury Vapour Flourescence (MBFRU, 400 watt., 250 volt) lights because at that time of the year the natural light inside the glasshouse is low. In order to have an idea of the light environment on the experiment bench, four calibrated quantum sensors connected to a data logger (21 × micrologger, Cambell Scientific Inc., Utah, USA) were placed on the bench diagonally along the line of variation of light and an hourly average photosynthetic photon flux density (μmol m^{-2} s^{-1}) was recorded from 13th February,1990 to 26th March,1990 (Fig. 5.1).

In addition, daily maximum and minimum temperature and relative humidity were recorded by a Thermohygrograph (Cassella, London, U.K., Fig.5.2) placed near the experimental bench.

Finally, after 85 days of growth, the plants were harvested on 4th April,1990 and the root, stem and leaf portions separated. Leaf area (cm^2) of each plant was determined by an Area Meter (L1-3100, Li-Cor, Lincoln, USA). The root, stem and leaf samples were oven dried at 70 °C for 7-10 days and their dry weight was recorded. The relative growth rate ($\overline{RGR}$) ,net assimilation rate ($\overline{NAR}$), leaf area ratio ($\overline{LAR}$), specific leaf area ($\overline{SLA}$) and leaf weight ratio ($\overline{LWR}$) were then calculated as described in chapter-4. Immediately before harvesting, an attempt was made to measure the rate of photosynthesis and stomatal conductance by using an ADC CO_2 gas analyser (The Analytical Development Co. Ltd., Hoddesdon, England) but rates were by that late stage very low as a result of stomatal closure, possibly because of root accumulation and compaction in the bottom of the pots . A possible explanation is suggested by work of Zhang and Davies (1989): that abscisic acid (ABA) produced by water-stressed roots is translocated in the traspiration stream to influence stomata, independently of any changes in leaf water status. This may have been the reason for the low stomatal conductance .

5.3 RESULTS

5.3.1 Effect of Salinity on Survival of Seedlings During Screening Test:

At the end of the experiment, the number of seedlings surviving in each tray was recorded and the survival percentage calculated (Table 5.1). The confidence limits for percentage data were calculated as suggested by Bailey (1959). In the control treatment,100% survived.

The survival percentage decreased significantly with increase in salinity (Table 5.1). Only about 55% of the plants survived when subjected to NaCl salinity at 102 mol m^{-3} whereas the survival under 205 and 410 mol m^{-3} was about 35% and 14% respectively.

TABLE 5.1 : Survival percentage of *E. microtheca* during screening test

Treatment	Survival percentage ± Confidence limit
Control	100
102 mol m^{-3}	55.1 ± 0.04
205 mol m^{-3}	34.7 ± 3.16
410 mol m^{-3}	13.7 ± 2.03

5.3.2 Effect of Salinity on the Morphology of the Seedlings

The effect of NaCl salinity on the morphology of seedlings became more evident as the number of applications of saline solution increased. Symptoms of salt injury started appearing after 6-8 applications of saline solution and were comparatively clear and conspicuous in higher levels of salinity.

The first effect observed was a change in colour of the leaves turning green to pale and then yellowish. This response appeared first in lower leaves, and proceeded progressively towards the top of the plant. At a later stage the tip and margins of the leaves started rolling inwardly, some of the leaves appeared burned and then wilted, and finally the leaves died and dropped.The progressive rolling , wilting and shedding of leaves from bottom to top of the plants,which was more intense in higher salinities, resulted ultimately in death.

5.3.3 Plant Mortality During Growth Trials

Although the less tolerant individuals within the population of *E. microtheca* were screened out during first stage of the study (screening test), some of the plants died when subsequently subjected to the highest level of salinity i.e. 410 mol m^{-3} during growth trials (Table-5.2). Mortality only occurred under the highest level of salinity (410 mol m^{-3}).

TABLE 5.2 : Mortality in *E. microtheca* during growth trials

Treatment	Number of plants that died (out of 7)
Control + 410 mol m^{-3}	6
210 mol m^{-3} + 410 mol m^{-3}	4
205 mol m^{-3} + 410 mol m^{-3}	3
410 mol m^{-3} + 410 mol m^{-3}	3

Highest mortality i.e. 6 plants (out of 7) was in plants from the control source when subjected to the highest level of salinity (410 mol m^{-3}) showing that these plants were not resistant genotypes. At the same time only 3 plants (out of 7) selected from highest salinity source died when given the same level of salinity . There was a decreasing trend in mortality of plants derived from increasing levels of salinity during the screening test.

5.3.4 Effect of Salinity on the Relative Growth Rate (RGR)

The mean relative growth rate of harvested plants among the population of *E. microtheca* , linearly decreased with increase in salinity as compared to the control (Fig.5.3.A), irrespective of source treatment. However, the RGR was also affected by source treatment, increasing in all (combinations of) treatments with increasing source salinity when compared with the corresponding control. In other words, the growth performance of plants selected from the highest salinity treatment during screening test was always better than that of plants derived from lower concentrations of NaCl.

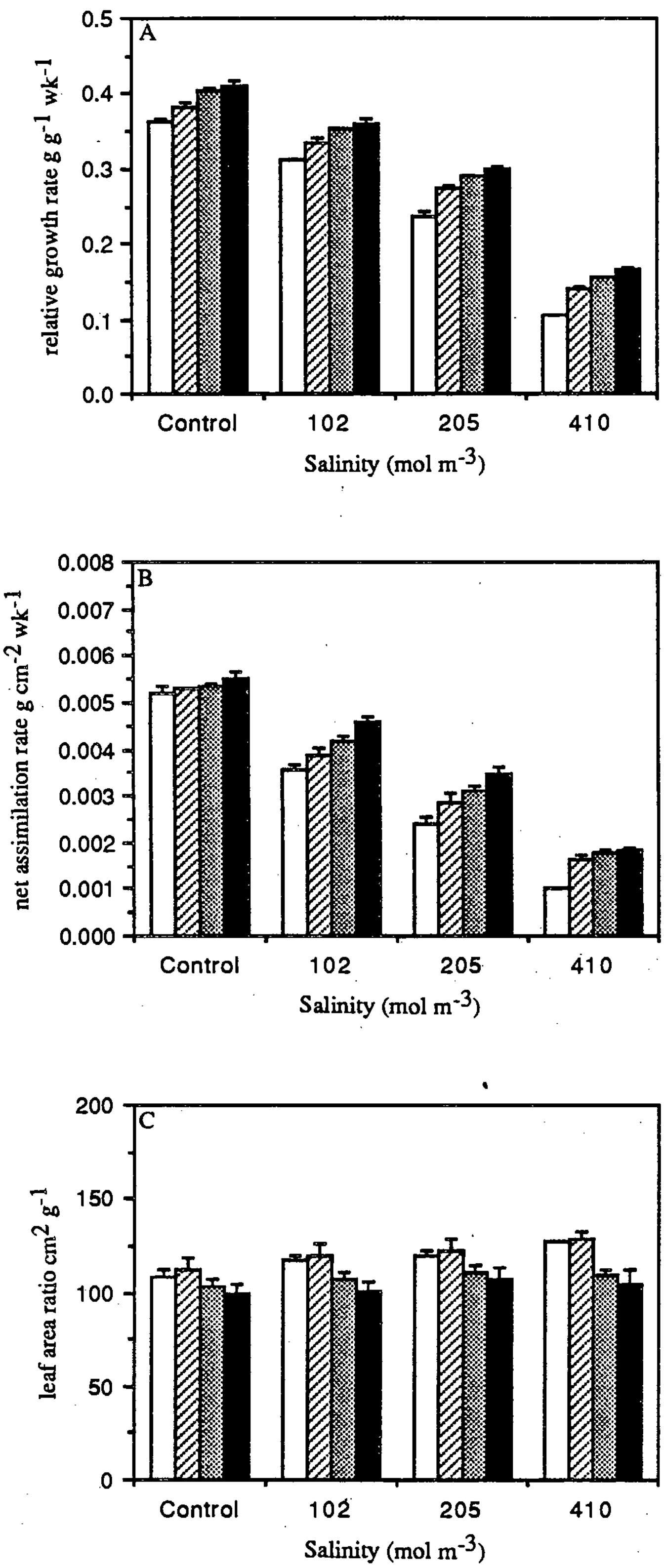

FIGURE 5.3: Relative growth rate, net assimilation rate and leaf area ratio of *Eucalyptus microtheca* grown under differant salinity regimes. The vertical bar indicates standard error of mean.The number of plants(n) in each case was seven except at 410 mol m^{-3} level of salinity where n=1,3,4 and 4 for control,102, 205 and 410 mol m^{-3} respectively.Columns indicate the treatments received during the initial screening: Control(□), 102 mol m^{-3}(▨) , 205 mol m^{-3}(▩) and 410 mol m^{-3}(■).

5.3.5 Effect of Salinity on Net Assimilation Rate (NAR)

Net assimilation rate (NAR) showed similar trends to RGR (Fig.5.3.B), decreasing with increase in salinity as compared to the control. However, contrary to RGR, there was a variable effect of source treatment on NAR (Fig. 5.3.B). Also, the NAR was effected by the source treatment in plants when treated with 102 and 205 mol m^{-3}. It increased under these two cocentrations of salt with increase in source salinity. The NAR of plants derived from control was decreased to a greater extent as compared to treated plants when subjected to highest level of salinity (410 mol m^{-3}).

5.3.6 Effect of Salinity on Leaf Area Ratio (LAR)

Leaf area ratio (LAR) was not significantly affected with increase in salinity but was significantly influenced by the source treatment (Fig.5.3.C). There was a trend of decrease in LAR with increase of salinity in source treatment although there was no significant difference between plants derived from the lowest level of salinity (102 mol m^{-3}) and control.

5.3.7 Effect of Salinity on Specific Leaf Area (SLA)

Similar to LAR, the specific leaf area (SLA) was not significantly affected with increase in salinity irrespective of source treatment (Fig. 5.4.A). There was a trend of decrease in SLA with increase in source treatment with the exception that there was no significant difference between plants derived from the lowest level of salinity (102 mol m^{-3}) and control. The plants derived from higher source salinity (205 & 410 mol m^{-3}) gave lower values of SLA then those derived from lowest level of salinity (102 mol m^{-3}) or control . This pattern of response is similar to LAR.

5.3.8 Effect of Salinity on Leaf Weight Ratio (LWR)

The leaf weight ratio (LWR) was not significantly affected either by salinity or source treatment (Fig. 5.4.B) .

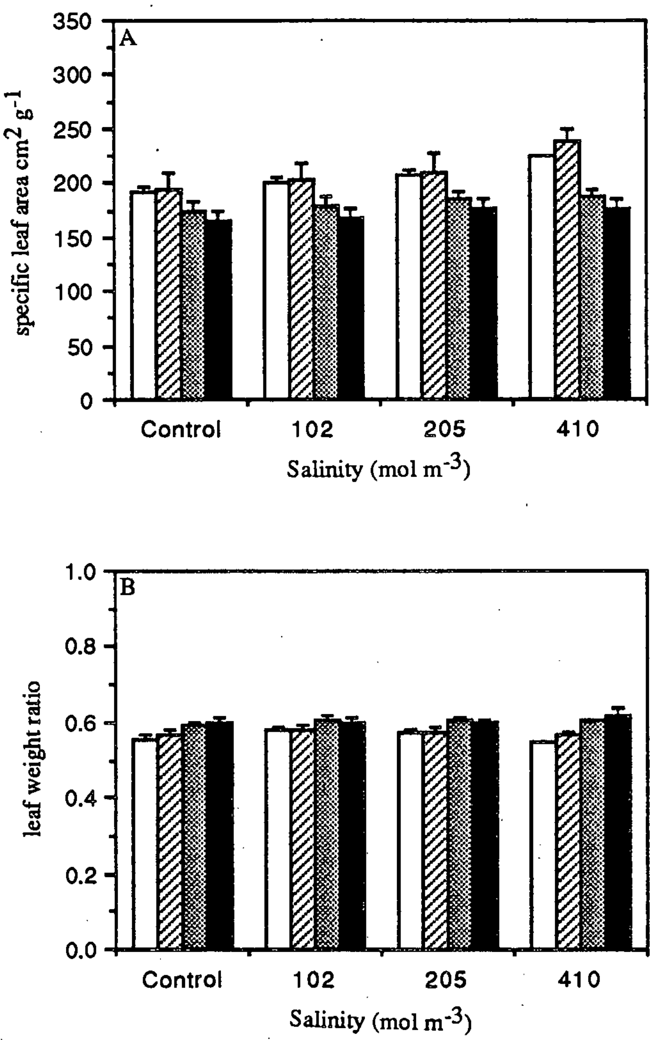

FIGURE 5.4: Specific leaf area and leaf weight ratio of *Eucalyptus microtheca* grown under different salinity regimes. The vertical bar indicates standard error of mean. The number of plants(n) in each case was seven except at 410 mol m^{-3} level of salinity where n=1,3,4 and 4 for control, 102, 205 and 410 mol m^{-3} respectively. Columns indicate the treatments received during the initial screening: Control(□), 102 mol m^{-3}(▨), 205 mol m^{-3}(▩) and 410 mol m^{-3}(■).

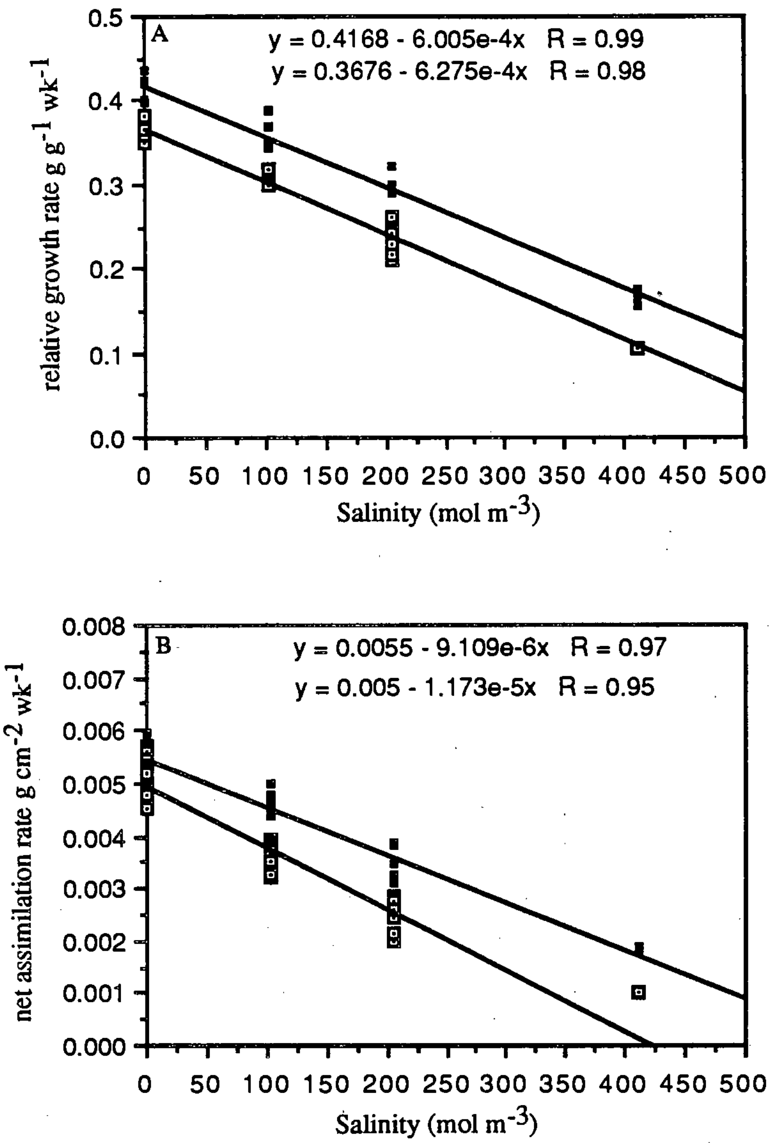

FIGURE 5.5: Relative growth rate and net assimilation rate of the selected(▪)(treated with 410 mol m^{-3} during screening test) and unselected(◘) plants of *Eucalyptus microtheca* grown under different salinity regimes.

5.4 DISCUSSION

The tolerance of a plant to salinity is a measure of its ability to withstand the effects of soluble salts concentrated in its root zone (Shannon, 1979) and is usually manifested through germination percentage, seedling survival and relative growth (Tal, 1985)

One of the main effects of salinity was a significant reduction in survival of *E. microtheca* (Table 5.1) which supports the findings of Sands (1981) and Radhi (1985) who also reported differences for salt tolerance in *E. camaldulensis*. The survival of some individuals at high concentration of salinity, strongly suggests the existence of genetic variation for salt tolerance within the population of the species (see also Blake,1981). The large variation in survival (55-14%) found within the population implies that the relative salt tolerance of a species is markedly affected by such variation and needs to be considered in comparison between species.

The results of the experiment reveal that salinity has a marked effect on net assimilation rate (NAR) and through it on relative growth rate (RGR) (Fig. 5.3.A &B). The NAR , an index of the efficiency of leaves as producers of new materials, was significantly decreased with increase in salinity and the differences in NAR between the salinity levels were substantial. The NAR is not a pure measure of photosynthesis: rather it depends on the excess of dry matter gain by photosynthesis over respiration integrated over the period of growth. Salinity may have influenced both photosynthesis and respiration . A higher respiration rate in salt-affected plants may have been the result of increased energy requirements in order to maintain an internal homeostasis (Shannon,1979).

Replotting the data of Fig. 5.3.A shows that there was a linear decline in RGR with increase in salinity (Fig. 5.5.A) and the growth analysis suggest that the NAR was almost exclusively responsible for the decrease in RGR with increase in salinity. The leaf area ratio (LAR) did not significantly contribute towards the decline in RGR. This is in sharp contrast to the case of Mustard (chapter 4).

Salinity is known to affect many aspects of plant metabolism (Poljakoff-Mayber, 1975) but it generally reduces the photosynthetic rate (Downton, 1977; Gale, 1975 and Nieman, 1962). Schwarz and Gale (1981) estimated that 76% of the growth reduction in *Xanthium strumarium* was related to reduced photosynthesis and the remainder due to increased maintenance respiration. Pezeshki and Chambers (1986) also reported a 72% decline in stomatal conductance and as much as 86% reduction in net photosynthesis in *Fraxinus pennsylvanica* as a result of application of saline water.

Also the chlorophyll content is affected by salinity and Longstreth *et al.*, (1984) reported a decrease in total chlorophyll in *Alternanthera philoxeroides* at all salinities.

Although in general the RGR decrease linearly with increase in salinity, the selected individuals differed from unselected ones in several aspects. The RGR of the selected individuals was higher than that of the unselected ones. However, the fact that selected individuals grow best even at zero salinity (Fig. 5.3.A) suggests that in the screening test there is selection for vigour *per se*.

Although the linear decline in RGR of selected plants is apparently less steep than that of unselected ones (Fig. 5.5.A) the difference in slope is not statistically significant and the two lines should be considered parallel. At the same time data of Fig. 5.3.B were also replotted (Fig. 5.5.B) and like RGR, the selected individuals differed in NAR from the unselected ones. The NAR decreased linearly with increase in salinity. The NAR of the selected plants diverged from the unselected plants with increase in salinity (Fig. 5.5.B). This type of response suggests that the leaves of selected plants were more able to maintain their rate of photosynthesis at high salinity. However, even the NAR in the selected plants was higher than the unselected at zero level of salinity (Fig. 5.5.B). These results suggest that selection of the individuals within the population of the *Eucalyptus microtheca* was both for their vigour and salt tolerance. It may be that the salt tolerant individuals are at the same time vigorous.

LAR was also affected by previous treatment and it decreased with an increase in salinity during the screening phase (Fig. 5.3.C). In general, plants avoid toxic salt effects either by restricting ion uptake (and then making necessary osmotic adjustments) or by allowing osmotic regulation through ion uptake and then adjusting to high salt concentrations in the green tissues. Actually, most plants are probably between these extremes and are restrictive accumulators of salts (Shannon, 1978). The reduction in leaf area per unit dry matter can therefore be attributed to accumulation of Na^+ & Cl^- ions in the leaves which results in reduction in elongation and division of cells (Waisel, 1972). In the present experiment the actual area of leaf exposed was restricted in the saline treatments by rolling of leaf tips and margins, particularly of lower leaves, more intense at higher salinity levels. It is probable that this was caused by the toxic effects of accumulated Na^+ and Cl^- in leaves. The way in which the plants died during the screening phase by first shedding their lower leaves may be an adaptive trait which leads to elimination of excess ions contained in dead leaves (Rush and Epstein,1981 and Yeo, 1983).

Radhi (1985) also found the accumulation of Na^+ and Cl^- in roots and leaves of those *E. camaldulensis* seedlings that suffered the greatest reduction in relative growth rate and leaf area.

The effect of previous treatment (during screening test) on LAR was because of changes in the specific leaf area (SLA) and not in the leaf weight ratio (LWR)(Fig. 5.4. A & B). The SLA of the selected plants was decreased with the exception that there was no significant difference between the plants derived from lowest salinity (102 mol m^{-3}) and control (Fig.5.4.A). SLA is dependent on leaf thickness, the relative proportions of different types of tissue and the packing of cells within the mesophyll. The data suggest that the leaves of the selected plants at 205 & 410 mol m^{-3} salinity became thicker as compared to unselected plants (Fig. 5.4.A). This increase in thickness of leaves is mainly due to the increased thickness of the whole mesophyll layer and particularly the palisade layer (Esau,1953 and Fahn,1967). The thickness of the leaf is affected by its water content and is related to the elasticity of the cell wall (Gardner and Ehlig, 1965). Such a response to NaCl salinity is well documented (see Meiri and Poljakoff-Mayber, 1967; Poljakoff-Mayber, 1975; Strogonov, 1964; Waisel, 1972 and Wignarajah *et al.*, 1975) and the induced thickness may help the plants to conserve water (Longstreth *et al.*, 1984).

Although differences in salt tolerance between varieties and origins of agricultural crops are extensively reported in the literature little appears to be known about such variation within (as compared to between) tree species. However, the limited evidence available in the literature suggests such variation for salt tolerance within the population of tree species does exist. Sands (1981) reported differences in salt tolerance between different provenances of *E. camaldulensis* and he proposed a comprehensive screening programme within that species.

Moezel *et al.*, (1988) reported a decline in growth of *E. camaldulesis, E. comitae-vallis, E. lesouefii, E. platycorys, E. spathulata and Casuarina obesa* in relation to salt. They obtained the required level of salinities by mixing NaCl, $MgSo_4$ and $CaCl_2$ to give Na: Mg: Ca ratio of 10: 2: 1 by weight. The salt concentration was increased weekly by 700 mS m^{-1}. Clemens *et al.*, (1983) by applying NaCl of 75 and 150 mM found depression of growth of 11 *Casuarina* species with increase in salinity. Radhi (1985) reported decrease in relative growth rate of *E. camaldulensis* with increase in salinity by applying 128, 275 and 575 mol m^{-3} NaCl solution. Growth investigations of *Acacia saligna* by Shaybany and Kashirad (1978) by applying 48, 96, 144, 192 and 240 mol m^{-3} of salinity revealed that almost all the growth parameters were reduced with increase in salinity.

In conclusion, the work described in this chapter shows that salt reduces the relative growth rate of *E. microtheca* mainly through an effect on net assimilation rate. The screening test showed that some individuals could grow even at 410 mol m^{-3} of NaCl. It was observed that they outperformed non-selected plants when compared in a saline medium, but they also outperform the non-selected plants when comparison is made without salt in the medium. It appears that selection has been for vigour and salt tolerance. The relationship between vigour and salt tolerance might be the subject of further study.

CHAPTER 6

EFFECT OF SALINITY ON THE GROWTH OF *Acacia nilotica (L.)* Willd. ex Del.

6.1 INTRODUCTION

Acacia nilotica is an indigenous tree of the Indian sub-continent. It is one of the most conspicuous trees in agricultural lands as well as marginal lands throughout Pakistan. Being a multipurpose tree with many end uses, as well as being easy to grow under difficult conditions, it has become a favourite among farmers. Its potential to grow on saline and alkaline soils is widely reported in the literature (Yadav, 1980; Khanduja *et al.*,1987; Singh *et al.*, 1986; Khan and Yadav, 1962 and Malik and Sheikh, 1983) but almost no work on its relative growth performance under different salinity regimes and variability within the species for salt tolerance has been done. The objective of this experiment were the same as experiment on *Eucalyptus* (5.1).

6.2 MATERIALS AND METHODS

Seeds of *Acacia nilotica* was obtained from Pakistan (it is not certain whether the seed was collected from one or more sources) and the experiment was set up in the glasshouse. Before sowing, seed was immersed in boiled water for 24 hours in order to obtain quick and higher germination (Troup, 1921 and National Academy of Sciences, 1980).

The seed was sown in plastic seed trays filled with the mixture of perlite and vermiculite mixed in equal volumes (1:1) on 31st October, 1989. Thirteen trays were sown with 104 seeds at a spacing of 2.5 cm x 2.5 cm over the full size of the tray. Six trays were sown with 57 seeds over half of each tray at the same density.

The seed started germinating after one week and on completion of germination Hoagland nutrient solution (1/10 strength) was applied daily, the concentration being gradually increased.

The Rhizobium strain USDA 3451 recommended for *Acacia* and *Prosopis* and obtained from Institute of Terrestrial Ecology at Bush, Edinburgh was first cultured in an autoclaved sterile mineral solution, then mixed in Hoagland nutrient solution and applied on 21st October, 1989 through irrigation to all trays except three of the half sown trays.

6.2.1 Screening Test

The design of the experiment for screening putative resistant genotypes within the population was a randomized block design with four blocks (replications) and three levels of NaCl concentrations (102, 205 and 410 mol m^{-3}). In order to detect a possible effect of inoculation, the control treatment was split into two, 'with' and 'without' Rhizobium.

In this way there were altogether 19 trays (13 full and 6 half sown) allotted to three levels of NaCl (12 full sown trays), control with Rhizobium (3 half and 1 full sown trays) and control without Rhizobium (3 half sown trays). Under these arrangements, there were no seedlings under 'control without Rhizobium' in block-4 because of lack of seedlings available.

The treatments within each block were assigned randomly. Before the start of treatments, the number of seedlings in each tray was recorded and the application of NaCl mixed in Hoagland solution was started on 4th December, 1989 when the seedlings were 20-25 days old. The trays were arranged on one half of a bench (2.75 $\times$ 1.83 m) in the glasshouse and were raised about 3 cm above the surface in order to avoid any chances of contamination of roots and to allow quick drainage of any excess water. The seedlings were irrigated daily with the three NaCl concentrations (102, 205 and 410 mol m^{-3}) mixed in Hoagland nutrient solution to achieve the desired concentration of salt in the media. It was assumed that the required level of salinity was achieved in the growth medium after 6 applications, as suggested by the results of the preliminary study (Fig. 4.1). The seedlings assigned to control treatment (both control and control + Rhizobium) were also irrigated daily with only Hoagland nutrient solution. The saline solution was added every time in sufficient amount to ensure that all solution in the medium was replaced and that the growth medium was kept at or close to field capacity all the time.

A slight effect of treatment was seen after 7 days in the form of yellowing and wilting of leaves particularly in seedlings treated with high concentration of NaCl, the effect becoming clear and conspicuous with time. The treatments continued till 20th, December, 1989 (treatment period = 17 days) when the differences between treatments were clear. The survival of seedlings under each treatment in each block was recorded.

The plants which died as a result of NaCl stress are considered intolerant individuals within the population of the *Acacia nilotica* and those which survived, supposedly putative resistant genotypes, were taken ahead for further investigations in the second stage of the experiment (6.2.2).

Daily maximum and minimum temperature and relative humidity were recorded (5.2.2 & Fig. 5.2).

6.2.2 Growth Trials

Uniform-sized plants surviving from the screening test (6.2.1) were transplanted from plastic trays into plastic pots (23.5 cm high × 8 cm diameter) perforated at the bottom to allow drainage. The pots were filled with the mixture of perlite and vermiculite mixed in equal volume (1:1). An examination of the roots at the time of transplanting suggested that the plants inoculated by Rhizobium had not been infected. Therefore once again the Rhizobium (USDA 3451) cultured in an autoclaved sterile mineral solution was applied to the roots at the time of transplanting, with a sterile polypropylene syringe. The Rhizobium was applied to all plants except those from control without Rhizobium. However, at the end of the experiment when plants were harvested, examination of the roots indicated that no nodulation had occurred.

All plants were irrigated with Hoagland nutrient solution (irrespective of the source treatment) and were allowed to grow until 9th January, 1990 so that they should fully recover from transplant shock.

The design of the experiment was a randomized block design with five blocks (replications) and three levels of NaCl concentrations (102, 205 and 410 mol m^{-3}). The control treatment was split, similar to the screening test, into 'control without Rhizobium' and 'control with Rhizobium'. For this part of the study (like the *Eucalyptus* experiment), all possible combinations of four current treatments and four source treatments were made giving the twenty combinations (page 74).

In this way, a total of 100 seedlings were used (one for each treatment combination × 5 blocks) in addition to 25 seedlings (5 seedlings from each source treatment) harvested for initial weights and leaf areas.

The treatments within the blocks were assigned randomly and the blocking pattern of screening test (6.2.1) was also maintained during this part of the study.

The pots with seedlings were placed in plastic trays which were raised 3 cm above the surface of the experimental bench in order to avoid contamination of roots and provide easy and quick drainage of any excess water or saline solution. The experiment was set up in a glasshouse on one half of the bench (2.75 m × 1.83 m) which is illustrated in plate- 2.

Source		treatment assigned
Control	+	Control
Control	+	102 mol m^{-3}
Control	+	205 mol m^{-3}
Control	+	410 mol m^{-3}
Control+Rhizobium (R)	+	Control+Rhizobium (R)
Control+R	+	102 mol m^{-3}+R
Control+R	+	205 mol m^{-3}+R
Control+R	+	410 mol m^{-3}+R
102 mol m^{-3}	+	Control
102 mol m^{-3}	+	102 mol m^{-3}
102 mol m^{-3}	+	205 mol m^{-3}
102 mol m^{-3}	+	410 mol m^{-3}
205 mol m^{-3}	+	Control
205 mol m^{-3}	+	102 mol m^{-3}
205 mol m^{-3}	+	205 mol m^{-3}
205 mol m^{-3}	+	410 mol m^{-3}
410 mol m^{-3}	+	Control
410 mol m^{-3}	+	102 mol m^{-3}
410 mol m^{-3}	+	205 mol m^{-3}
410 mol m^{-3}	+	410 mol m^{-3}

The treatments were started on 9th January, 1990. The plants were irrigated with saline solution daily in an amount sufficient to ensure that all the previous solution was replaced by fresh solution and that the medium was maintained at or close to field capacity.

The solution that leached from the pots (3 pots chosen at random for each treatment combination) was collected, first after one week and then after ten days. The electrical conductivity of this leachate was measured (as explained in 5.2.2) which indicated that after 6-7 applications of the saline solution the electrical conductivity of the leachate was the same as that of the saline solution applied, thereafter staying constant. Thus, we can be sure that the desired level of salinity in the growth medium was achieved.

The light environment on the experimental bench, the temperature and the relative humidity during this part of the study were the same as in the *Eucalyptus* experiment (Fig. 5.1 & 5.2).

Plate 2: Growth trials of *Acacia nilotica* set-up in glasshouse as a randomized block design illuminated with Mercury Vapour Fluorescence lamps.

Finally, after 57 days of growth the plants were harvested on 6th March, 1990 and the root, stem and leaf portions were separated. Leaf area was measured by an Area Meter as in the earlier experiment on *Eucalyptus* (5.2.2). The root, stem and leaf samples were oven dried at 70 ^{0}C for 7-10 days and their dry weights were recorded. The leaflets were separated from petioles in order to determine the dry weight of the leaf lamina as suggested by Medina (1984).

The mean relative growth rate ($\overline{RGR}$), net assimilation rate ($\overline{NAR}$), leaf area ratio ($\overline{LAR}$), specific leaf area ($\overline{SLA}$) and leaf weight ratio ($\overline{LWR}$) were then calculated as described in chapter-4 (4.2.3)

6.3 RESULTS

6.3.1 Effect of Salinity on Survival of Seedlings During Screening Test

At the end of the screening test, the number of seedlings surviving in each tray was recorded and the survival percentage calculated (Table 6.1): Confidence limits were calculated as in the *Eucalyptus* experiment (5.3.1).

Survival was 100% under control treatments, decreasing significantly with increase in salinity (Table 6.1). The survival percentage of plants at the lowest level of NaCl concentration (102 mol m^{-3}) was 74% whereas it decreased to 52% and 8% at 205 and 410 mol m^{-3} respectively.

TABLE 6.1 : Survival percentage of *Acacia nilotica* during screening test

Treatment	Survival percentage ± Confidence limit
Control	100
Control + Rhizobium	100
102 mol m^{-3}	74.2 ± 7.4
205 mol m^{-3}	51.8 ± 8.5
410 mol m^{-3}	7.8 ± 4.7

6.3.2 Effect of Salinity on the Morphology of Seedlings

There was no effect of NaCl salinity on the morphology of seedlings till the seventh day of the treatment. After that, slight effects in the form of yellowing and burning of leaf tips and margins was observed particularly in plants treated with higher concentrations of salt.

With increase in the number of applications of saline solution the injury symptoms became clear. It was observed that first the lower leaves were affected by salinity through a change in colour from green to pale and then they became yellowish. At a stage of severe injury the lower leaves were first burned and wilted and finally shed. In this way the wilting and dropping of leaves progressively proceeded from the bottom of the plant to the top. The differences in severity of the salt injury were most obvious in higher levels of salinity.

6.3.3 Plant Mortality During Growth Trials

After the screening out of intolerant out individuals within the population of *Acacia nilotica* during the first stage of the study (6.2.1), the plants which survived were subsequently subjected to different salinity levels (6.2.2) during growth trials. Some of the plants still died when subjected to highest level of NaCl concentration (410 mol m^{-3}). Mortality occurred only under the highest salinity (Table 6.2). It implies that screening of intolerant individuals still occurred beyond stage 1 during growth trials at this concentration of salt.

TABLE 6.2 Mortality in *Acacia nilotica* during growth trials at the highest salinity

Treatment	Number of plants died (out of 5)
Control + 410 mol m^{-3}	4
Control + Rhizobium + 410 mol m^{-3}	5
102 mol m^{-3} + 410 mol m^{-3}	3
205 mol m^{-3} + 410 mol m^{-3}	3
410 mol m^{-3} + 410 mol m^{-3}	5

All the plants selected from Control + Rhizobium and 410 mol m^{-3} sources died when subjected to 410 mol m^{-3} NaCl salinity. This suggests that these plants were not true resistant genotype and the *Acacia nilotica* is not resistant to such a highest level of salinity (410 mol m^{-3}) in the first instance. However, some of the individuals taken from other source treatment (during screening test) (e.g. 102 & 205 mol m^{-3}) were able to survive even at the highest level of salinity (410 mol m^{-3}).

6.3.4 Effect of Salinity on Relative Growth Rate (RGR)

The mean relative growth rate (RGR) of harvested plants decreased with the increase in salinity as compared to control (Fig. 6.1.A) irrespective of source treatment during screening test (6.2.1). Nevertheless, the RGR was also affected by the source treatment (during screening test), increasing in all treatments with increase in source salinity compared with the corresponding control. But there was no difference in RGR of plants treated with lowest level of salinity (102 mol m^{-3}) during screening test. At the same time there was no difference in RGR of control plants grown with or without Rhizobium.

The growth of plants treated with the two higher salinity levels (205 & 410 mol m^{-3}) during screening test was always better than the plants taken from control or lowest salinity level (102 mol m^{-3}).

6.3.5 Effect of Salinity on Net Assimilation Rate (NAR)

There was a similar trend (like RGR) in net assimilation rate (NAR)(Fig. 6.1.B). NAR decreased with increase in salinity, irrespective of source treatment as compared to corresponding control. However the source treatment (during the screening test) has also effected the NAR, which increased with increase in source salinity, except that there was no difference in NAR between the plants selected from control treatment and lowest concentration of NaCl (102 mol m^{-3}).

6.3.6 Effect of Salinity on Leaf Area Ratio (LAR)

Leaf area ratio (LAR) was not significantly affected by an increase in salinity (Fig. 6.1.C). There was a decreasing trend in LAR with increase in source salinity

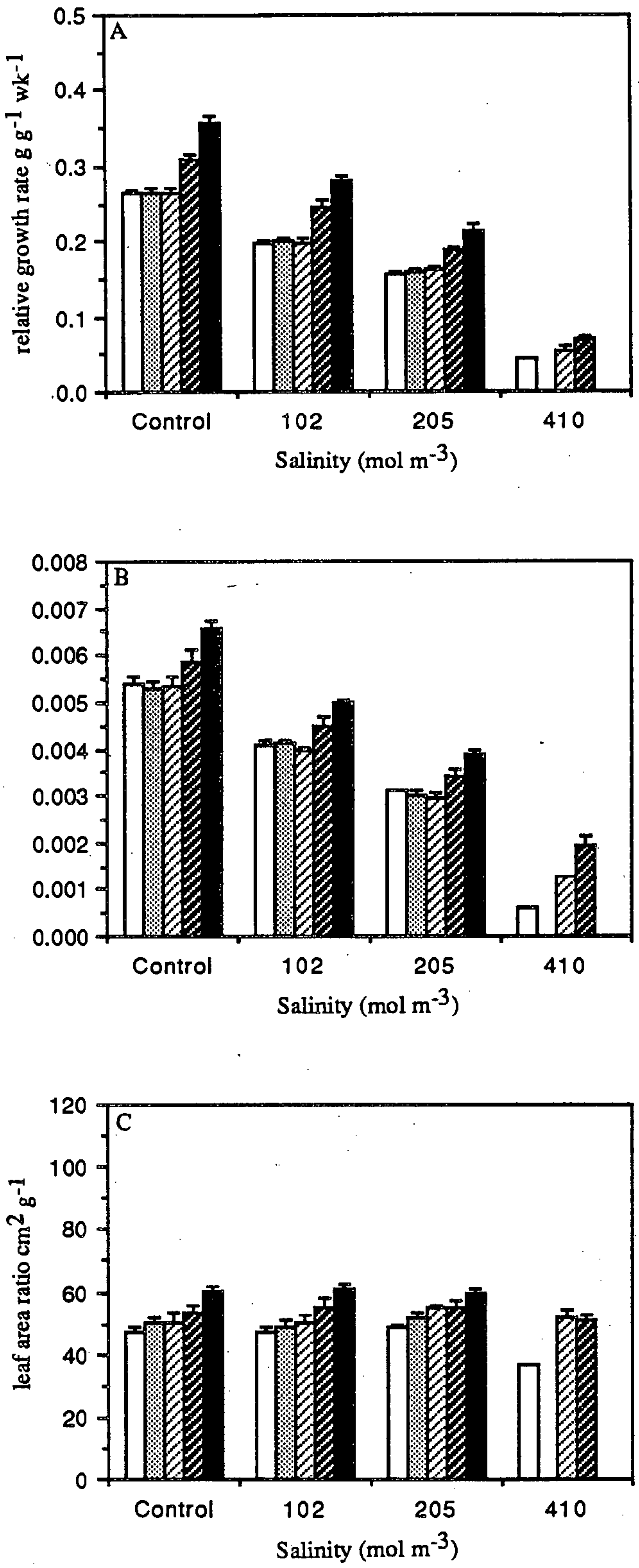

FIGURE 6.1: Relative growth rate, net assimilation rate and leaf area ratio of *Acacia nilotica* grown under different salinity regimes. The vertical bar indicates standard error of mean. The number of plants (n) in each case was five except at 410 mol m^{-3} level of salinity where n=1,0,2,2 and 0 for Control, Control+rhizobium, 102, 205 and 410 mol m^{-3} respectively. Columns indicate the treatments received during the initial screeing: Control(□), Control+rhizobium(▩), 102 mol m^{-3}(▨), 205 mol m^{-3}(▨) and 410 mol m^{-3}(■).

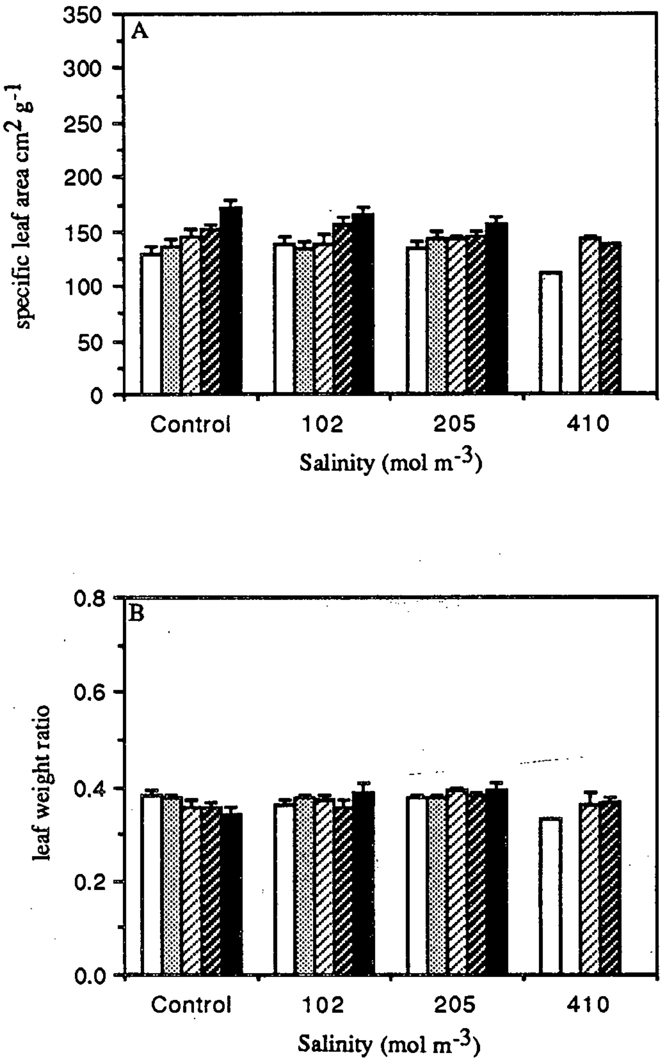

FIGURE 6.2: Specific leaf area and leaf weight ratio of *Acacia nilotica* grown under different salinity regimes. The vertical bar indicates standard error of mean. The number of plants (n) in each case was five except at 410 mol m^{-3} level of salinity where n=1,0,2,2 and 0 for Control, Control+rhizobium, 102, 205 and 410 mol m^{-3} respectively. Columns indicate the treatments received during the initial screening: Control(□), Control+rhizobium(▩), 102 mol m^{-3}(▧), 205 mol m^{-3}(▧) and 410 mol m^{-3}(■).

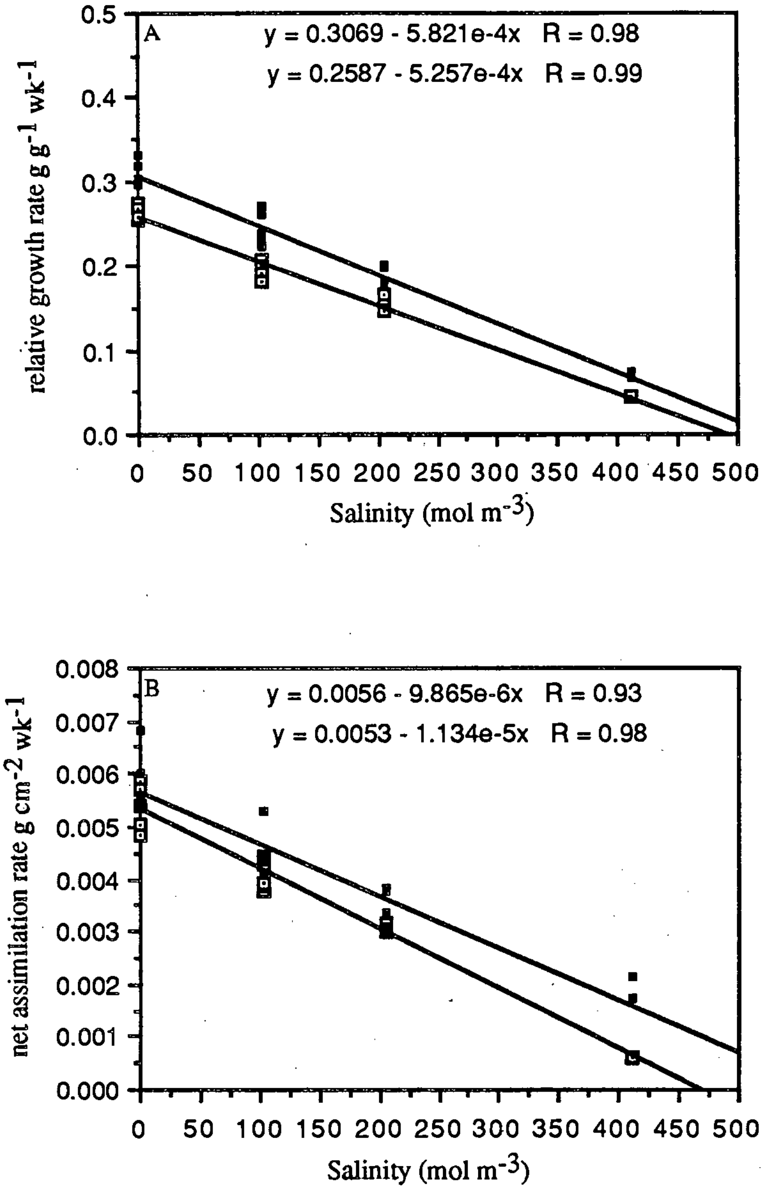

FIGURE 6.3: Relative growth rate and net assimilation rate of the selected(■)(treated with 205 mol m^{-3} during screening test) and unselected(◘) plants of *Acacia nilotica* grown under different salinity regimes.

(during screening test) but the only significant increase in LAR was obtained in plants derived from the highest level of salinity (410 mol m^{-3}) as compared to control. The plants treated with the two lower levels of salinity (102 & 205 mol m^{-3}) during screening test showed no significant difference in LAR when compared to control (Fig. 6.1.C).

6.3.7 Effect of Salinity on Specific Leaf Area (SLA)

There was a trend in specific leaf area (SLA) similar to LAR (Fig. 6.2.A). SLA was not affected by an increase in salinity. But the plants treated with the highest level of salinity (410 mol m^{-3}) during screening test showed an increase in SLA whereas there was no significant difference between those derived from the two lower salinity sources (102 & 205 mol m^{-3}) and the control (Fig. 6.2.A).

6.3.8 Effect of Salinity on Leaf Weight Ratio (LWR)

The leaf weight ratio (LWR) was not significantly affected by an increase in salinity in either first (screening) stage or the second (growth) stage (Fig. 6.2.B).

6.4 DISCUSSION

The results of this experiment are similar to those of the earlier study on *Eucalyptus* (chapter 5). The survival percentage significantly decreased with increase in salinity. At the highest level of salinity (410 mol m^{-3}) only 8% of the total population survived, whereas at the lowest salinity (102 mol m^{-3}) the survival percentage was 74%. The presence of some survivors at high salinity levels suggests the existence of genetic variability within the population of the species for their tolerance to salt.

The plants which survived as a result of the screening test under the highest salinity (410 mol m^{-3}) and were subsequently subjected to same level of salinity (i.e. 410 mol m^{-3}), could not withstand the treatment and died (Table 6.2), which implies that these individuals were not truly salt tolerant. The elimination of the treatment (410 mol m^{-3} + 410 mol m^{-3}) suggests that *Acacia nilotica* is not tolerant to such a high concentration of NaCl. It is most likely that if application of saline solution during the screening test had been continued for a longer period , those individuals would have died during the screening test.

At the same times some of the plants which were treated with the lower levels of salinity (i.e. 102 & 205 mol m^{-3}) during the screening test, were able to withstand the highest salinity (410 mol m^{-3}) during the subsequent growth study (Table 6.2). The low salt treatments during screening evidently has some effect on salt tolerance of plants in terms of survival.

One of the main effects of salinity was to decrease in net assimilation rate (NAR) (Fig.6.1.B). This resulted in a decline in relative growth rate (RGR) with salinity (Fig.6.1.A). There was no significant contribution by the leaf area ratio (LAR) (Fig. 6.1.C). The decline in RGR was caused by NAR and not LAR.

Non-halophytes exposed to a saline medium experience both a decline in water potential and an increased ion content in their tissues (Curtis and Lauchli, 1986). Osmotic adjustments and turgor maintenance can be achieved by the uptake of inorganic ions (Milford *et al.*, 1977; Downton and Loveys, 1981 and Walker *et al.*, 1983) and this can also lead to problems in ion compartmentation and a decline in leaf function (Downton and Millhouse, 1983 and Kingsbury *et al.*, 1984).

In this experiment, the efficiency of leaves as producers of new materials (i.e. NAR) was significantly affected by the increase in salinity. The NAR is the dry matter gain through photosynthesis over respiration on leaf area basis. A higher rate of respiration in salt-affected plants, might be due to increase in energy requirements in order to maintain an internal homeostasis (Shannon, 1979). Among the other effects of soil salinity, reduction in rate of photosynthesis is widely reported in the literature (Downton, 1977; Gale, 1975 and Nieman, 1962) and the result of this experiment support the work of the others.

Replotting of data in Fig. 6.1.A reveals that there was a linear decline in RGR with increase in salinity (Fig. 6.3.A). The decrease in RGR was caused by a reduction in NAR (Fig.6.1.B). LAR was not significantly effected by salinity (Fig. 6.1.C) nor were the components of LAR, the SLA and LWR (Fig. 6.2. A & B). However, the source treatment had an effect on RGR. The RGR increased with the increase in source salinity with the exception that there was no difference between the plants treated with the lowest level of salinity (102 mol m^{-3}) and the corresponding control (Fig.6.1.A). The increase in RGR was found in plants treated with 205 and 410 mol m^{-3} NaCl during the screening test. The source salinity has its effect beyond 102 mol m^{-3} salinity and effected NAR similarly to RGR (Fig.6.1.B).

The fact that selected individuals grew better at <u>zero</u> salinity than the corresponding control plants suggests that the selection was for vigour *per se* and this is further substantiated by the fact that the two lines in Fig. 6.3.A do not diverge at all, if any thing they converge.

The data in Fig. 6.1.A & B replotted in the form of Fig. 6.3.A & B revealed that the selected individuals differed in RGR and NAR from the unselected ones and the RGR and NAR of selected plants are higher than the unselected plants. Moreover, the NAR of the selected individuals diverged with increase in salinity from the unselected ones (Fig. 6.3.B). It implies that the leaves of the selected plants were more able to maintain a higher rate of gain in dry matter through photosynthesis over respiration. However, even the NAR in selected plants at zero salinity was higher than the unselected (Fig. 6.3.B). This suggest that the selection of individuals within the population of *Acacia nilotica* was not only for vigour but also for salt tolerance.

Although there was an increasing trend in LAR and SLA with increase in source salinity but it does not seems to be significant.

In summary, the results of this experiment show a clear response of *Acacia nilotica* to NaCl salinity. The progressive decrease in survival percentage suggests the existence of variability for salt tolerance within the population. Salt reduced the relative growth rate of the seedlings primarily through net assimilation rate. Although unselected individuals could not survive at 410 mol m^{-3} but the plants treated with 102 and 205 mol m^{-3} during the screening test were able to grow even at 410 mol m^{-3}. The selected individuals showed higher RGR and NAR as compared to the unselected seedlings. The results of the experiment suggest that the selection of plants within the population of *Acacia nilotica* was both for vigour and salt tolerance. The relationship between vigour and salt tolerance might be the subject of further study.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 GENERAL DISCUSSION

In the first section, some of the problems faced during the investigation are discussed and in the latter part the comparison of species studied and the practical implications of the results are considered.

The salt tolerance in agricultural crops has been studied by several workers but very little or no work has been done on trees. Some of the tree species, known to be salt tolerant, have been investigated but very little is known about the genetic variability within the population of a species. However, the first step in this direction is to evaluate the genetic variation for salt tolerance within the species and to develop a technique to identify and select resistant genotypes for outplanting. Some workers have found differences in salt tolerance among the provenances of a species and recommended a screening programme (e.g. Sands,1981 and Omran, 1986).

The aim of the present work was to develop a screening technique through which the putative resistant genotypes within a tree species could be identified and selected at an early stage of growth for planting in the field.

At the development stage of the study *Sinapis alba* was selected to work with, being widely available and fast growing. Subsequently this was followed by work on *Eucalyptus microtheca* and *Acacia nilotica*.

The seedlings were grown in an artificial substratum consisting of vermiculite (medium grade) and perlite (expanded volcanic rock) mixed in equal volume (1:1). Vermiculite, having larger absorbent area and more water retaining capacity, was used to keep the medium all the time at the required concentration of salt. Perlite was mixed with the vermiculite in order to avoid any waterlogging and create ample aeration, and this also facilitated the separation of roots without any loss when the plants were harvested.

Nonetheless, a difficulty was faced in establishing the required level of salinity in the growth medium, because it was not possible to prepare a soil extract of the artificial medium for measurement of electrical conductivity. The results of the preliminary study (Fig. 4.1) revealed that after 6 daily applications of saline solution, the electrical conductivity of the leachate was the same as that of the applied solution.

Thus, the required level of salinity in the media could be ensured by measuring the electrical conductivity of the leachate.

The plants which survived in a saline environment were considered to be salt tolerant due to their ability to withstand the stress, to germinate and grow to the seedling stage under such conditions (Hayward and Wadleigh, 1949; Richard, 1969 and Shannon, 1984). Therefore, survival under each level of salinity was considered a suitable criterion for identification and selection of putative resistant genotypes which were taken ahead for further investigation of their growth performance.

It seems likely that the medium and irrigation presented the roots with a uniform salinity, so it is unlikely that some plants survived because they occupied 'islands' of low salinity, as could occur in the natural environment.

In order to evaluate the growth performance of the selected individuals, they were subsequently subjected to different levels of salinity and the classical approach to plant growth analysis (Hunt, 1982) was used. The time-averaged values of whole plant relative growth rate ($\overline{RGR}$), net assimilation rate ($\overline{NAR}$), leaf area ratio ($\overline{LAR}$), specific leaf area ($\overline{SLA}$) and leaf weight ratio ($\overline{LWR}$) were derived to obtained a quantitative description of biomass accumulation and its relationship to leaf growth at each level of NaCl concentration. These methods, while having been used in a variety of contexts (reviewed in Hunt, 1982) have not been fully applied in the past to tree growth under saline conditions.

Acacia nilotica (sub family Mimoseae) is a nitrogen-fixing tree (Faria *et al*., 1989) and an attempt was made to inoculate its roots with the Rhizobium. But despite these efforts, *Acacia nilotica* seedlings could not be infected. Another attempt was made to measure the rate of photosynthesis and stomatal conductance in *Eucalyptus microtheca* with a CO_2 gas analyzer but the rates were by that time very low as a result of stomatal closure, possibly because of root accumulation and compaction in the bottom of the pot.

7.1.1 Comparison of Species

The comparison of Tables 5.1 and 6.1 reveals that the survival percentage of *Acacia nilotica* at all levels of salt was higher than *Eucalyptus microtheca* except at 410 mol m^{-3}. The survival of all three species, *Sinapis alba* (Figure 4.2), *Eucalyptus microtheca* (Table 5.1) and *Acacia nilotica* (Table 6.1) decreased with increase in salinity. The progressive decline in survival with increase in salinity suggests the existence of genetic variability for salt tolerance within the species. In *Acacia nilotica* only 8% of the population could survive at the highest level of salinity (410 mol m^{-3}) and they also subsequently died when again subjected to the same level of salt (see Table 6.2). Perhaps different stages of the life history display different sensitivity to salt. On the other hand, the survival in *Eucalyptus microtheca* seedlings at that level of salt (410 mol m^{-3}) was 14%.

Fig. 7.1 and 7.2 illustrate the main responses of the three species studied to NaCl salinity. One of the main similarities in the results given by the three species is a reduction in relative growth rate with increase in salinity (Fig. 7.1.A). In *Sinapis alba*, the relative growth rate decreased sharply with an apparent threshold for growth at 350 mol m^{-3} whereas for *Eucalyptus* and *Acacia* the thresholds apparently exceed 500 mol m^{-3}. At low salt concentrations *Sinapis alba* grows faster.

The reduction in relative growth rate (RGR) of the *Sinapis alba* was through reduction in leaf area ratio (LAR) (Fig. 7.1.B & C) whereas the RGR of *Eucalyptus microtheca* and *Acacia nilotica* was affected by the net assimilation rate (NAR) instead of LAR (Fig. 7.1.B & C). This is an intriguing discovery, and it would be interesting to extend the approach to more species to see whether all salt tolerant species behave like *Eucalyptus* and *Acacia*.

The LAR of *Sinapis alba* was reduced with increase in salinity, which resulted in decrease in RGR, and both specific leaf area (SLA) and leaf weight ratio (LWR) equally affected the LAR (Fig.7.2.A & B)

As far as the effectiveness of the screening technique is concerned, the selected individuals within the populations of *Eucalyptus microtheca* and *Acacia nilotica* showed better growth performance in terms increasing RGR as compared to the corresponding control during the growth trials (Fig. 5.3.A & 6.1.A). The trend of increase in RGR was true for all the treatments combinations including control with the exception that there was no difference in RGR of the plants of *Acacia nilotica* treated with the lowest level of salinity (102 mol m^{-3}) and the control (Fig 6.3).

The increase in RGR of the selected plants of both the species was through an increase in NAR. Fig. 5.5.B and 6.3.B describe that the leaves of the selected plants were able to maintain a relatively high rate of gain in dry matter through photosynthesis over respiration than the unselected ones. It suggests that the selection of individuals within the two tree species was for vigour and salt tolerance, as the selected individuals grew faster even at zero salinity, and the relative growth rates at high salinity were no less reduced in the selected individuals than they were in the unselected.

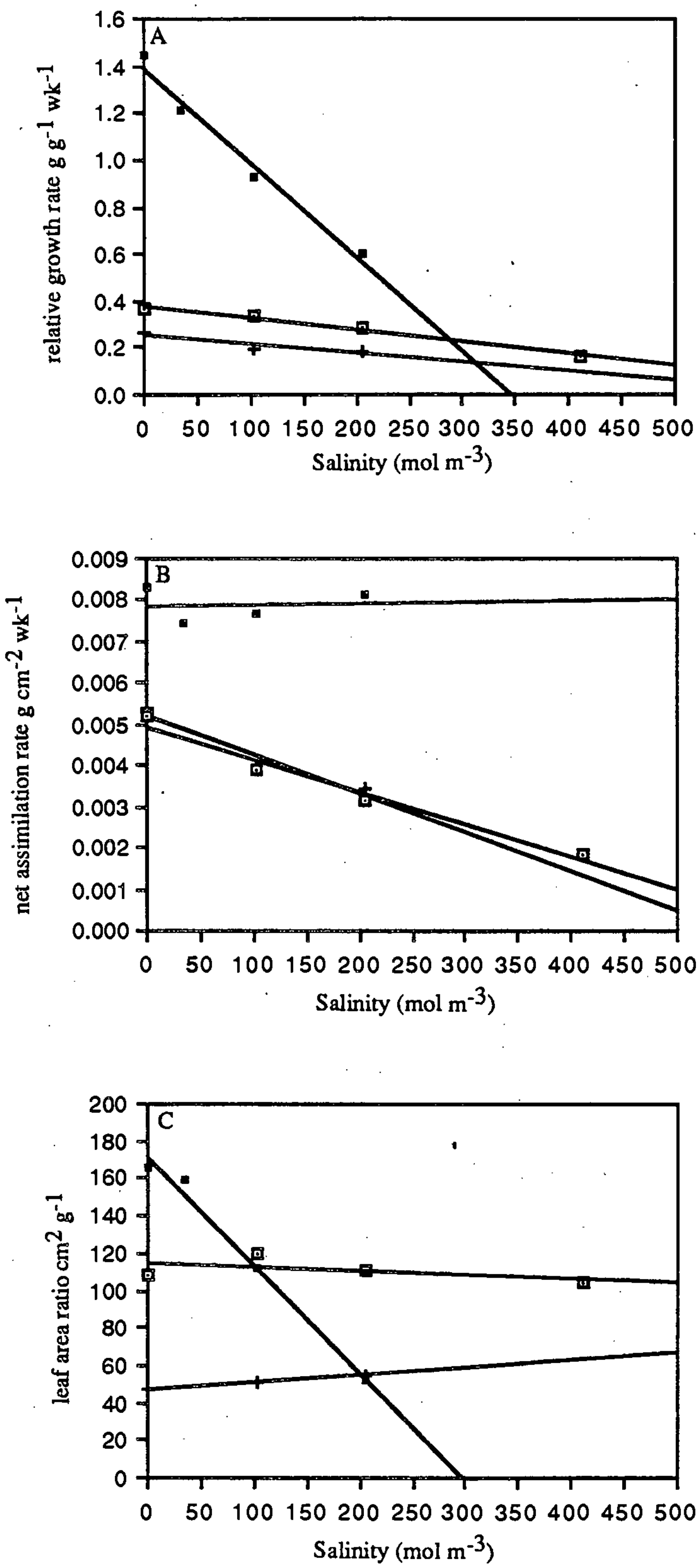

FIGURE 7.1: Relative growth rate, net assimilation rate and leaf area ratio of *Sinapis alba* (▪), *Eucalyptus microtheca* (◘) and *Acacia nilotica* (+) grown under different salinity regimes. The concentration of source salinity during screening tests was same of the NaCl solution applied during growth trials for all the three species to make them comparable.

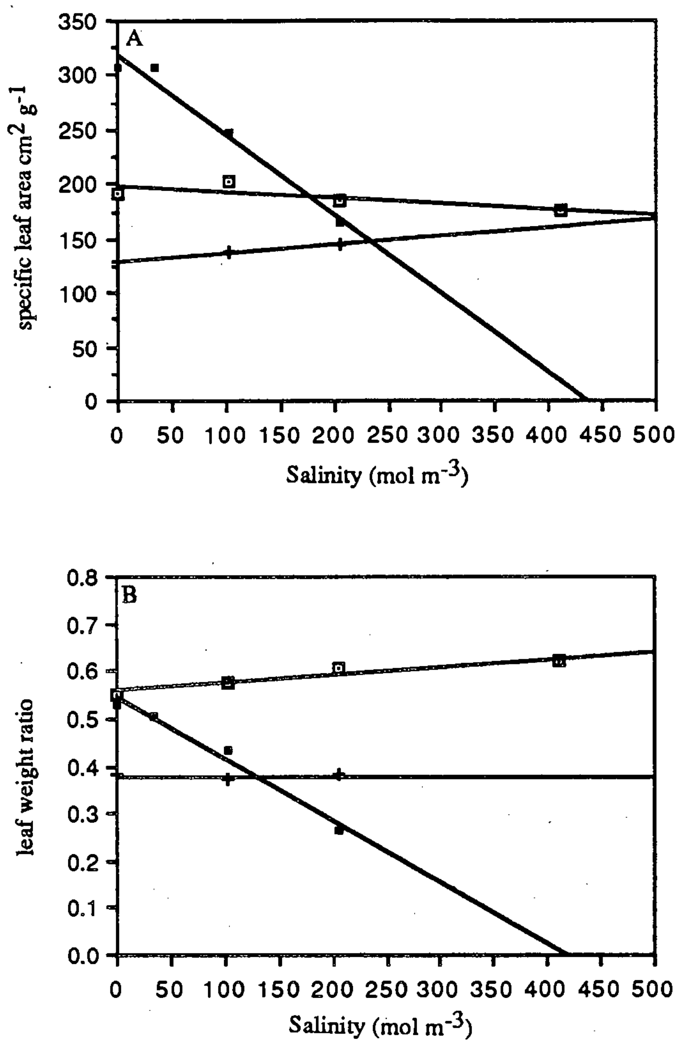

Figure 7.2: Specific leaf area and leaf weight ratio of *Sinapis alba* (▪), *Eucalyptus microtheca* (▣) and *Acacia nilotica* (+) grown under different salinity regimes. The concentration of source salinity during screenung tests was the same of the NaCl solution applied during growth trials for all the three species to make them comparable.

7.1.2 Practical Implications of the Results

In most of the cases, the nursery-raised seedlings are planted in the field and the choice of species includes consideration of all the ecological and socio-economic factors of a particular site. With the technique used here it is possible to select individuals within the population of the species which have the ability to withstand salt stress by being vigorous. An indication of increase in biomass production that might be expected is shown in Fig 7.3. Here the biomass is calculated from the RGR, assuming it remains constant over 25 weeks.

Through this technique less intolerant individuals within a species can be screened out at the nursery stage and in this way the expense of transportation, handling, planting and protection of these intolerant plants when planted in the field can be saved. In the future, it may be possible to add genetic tolerance to salt by the application of new genetic techniques. For example, a gene for salt tolerance located in another crop such as wheat, could be transferred to *Eucalyptus*. This might result in further benefits, but a stage of selection would still be useful because of that benefits of vigour shown in this study.

7.2 CONCLUSIONS

1. The presence of survivor at high salinity suggests the existence of genetic variability within the population. The survival percentage progressively decreased with increase in salinity.

2. One of the main effects of salinity was a linear reduction in relative growth rate with increase in salinity. There was sharp decline in RGR of the *Sinapis alba* whereas it decreased gradually in the two tree species.

3. The reduction in relative growth rate in *Sinapis alba* was through a decrease in leaf area ratio whereas in both the tree species (*Eucalyptus microtheca* and *Acacia nilotica*) it was through a reduced net assimilation rate.

4. Selected individuals displayed higher RGRs' than unselected individuals when comparison was made at both high and low concentrations of salt. In this way the gain in biomass production came through the selection of more vigorous individuals.

5. In *Sinapis alba* the decrease in leaf area ratio was through specific leaf area (SLA) and leaf weight ratio(LWR) whereas in the two tree species salinity has no affect on SLA and LWR.

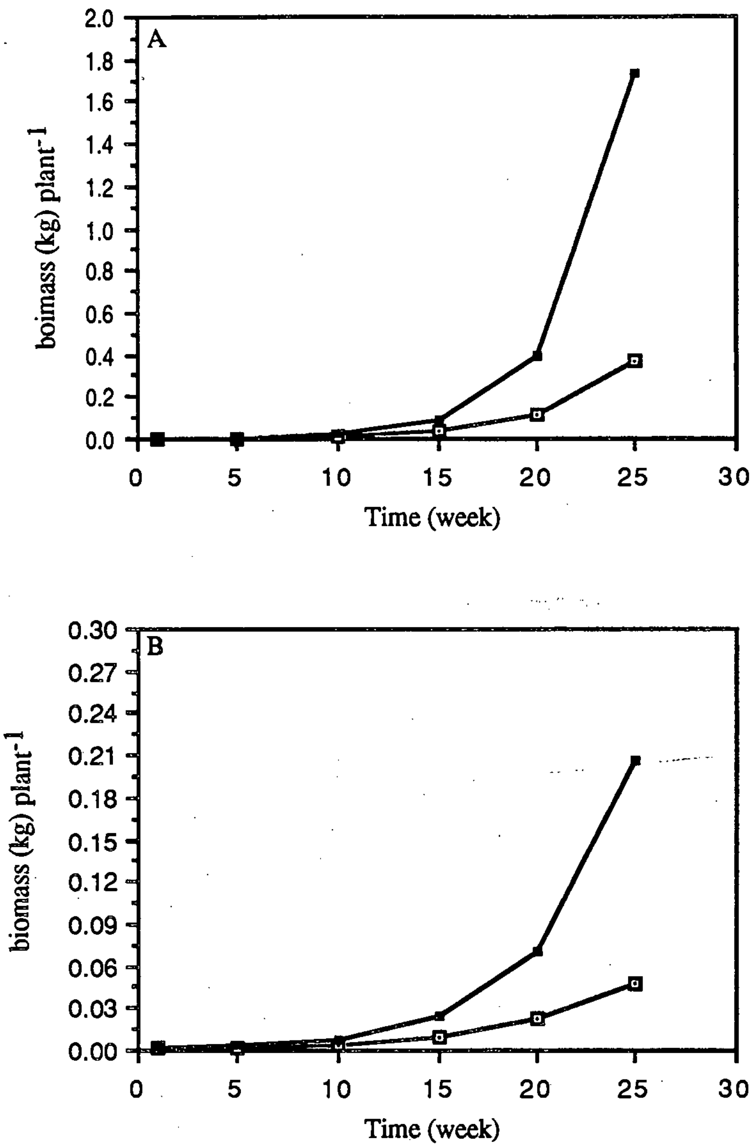

FIGURE 7.3: Expected biomass production of selected (▪) and unselected (◻) seedlings of *Eucalyptus microtheca* (A) and *Acacia nilotica* (B) when grown at 205 mol m^{-3} NaCl concentration with an initial biomass of one gram assuming the same rate of relative growth for each species during the period.

6. The source salinity (during screening test) does, however, have an effect on SLA of two tree species and through this on LAR.

7. *Eucalyptus microtheca* and *Acacia nilotica* showed high tolerance to NaCl salinity and seem to be suitable for planting on salt-affected areas.

8. It is possible through the screening technique to identify correctly and select vigorous and salt tolerant individuals within the population of the tree species.

7.3 SUGGESTIONS FOR FURTHER WORK

1. Field trials to elucidate the interaction of other factors and their effects on salt tolerance of the species are necessary.

2. Further studies are required to be conducted to investigate the physiological phenomena including rate of photosynthesis, stomatal conductance, accumulation of ions in different parts of the plant and rate of transpiration for higher salt tolerance in the selected individuals to see how the osmotic adjustments are achieved in these plants and what biochemical factors are responsible for higher relative growth rates.

3. The relationship between salt tolerance and vigour is unclear, possibly, fast growth provides greater possibility of 'diluting' the salt which is taken up. This requires investigation.

4. The study on anatomical differences of the selected and unselected plants would provide valuable information to understand the physiological basis for better growth performance.

5. The possibility of further propagation of the selected individuals through vegetative means should be explored.

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